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ERRATA AND AUTHORS' EMENDATIONS

Page 149, line 9, should read "The optimum reaction for growth" instead of "The optimum temperature for growth."

Page 360, line 15 from bottom, should read "rhabditiform" instead of "rhabitdiform."

Page 414, line 13, should read "Old cankers" instead of "Old ankers."

Page 464, line 9 from bottom, should read "respectively" instead of "respective."

Page 467, line 35, should read "than are preexisting" instead of "then preexisting."

Page 353, line 12 from bottom, should read "an organism endemic in nature" instead of "an organism more or less endemic."

JOURNAL OF AGRICULTURAL RESEARCH

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WORK AND PARASITISM OF THE MEDITERRANEAN FRUIT FLY IN HAWAII DURING 1919 AND 1920[1]

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Control by parasites has been the only method of combating the Mediterranean fruit fly (*Ceratitis capitata* Weidemann) in Hawaii that has met with any degree of success since its introduction in 1910. In 1911 the Territorial government inaugurated a clean-culture campaign, which was taken over by the Federal Bureau of Entomology in 1912 and continued until 1914. This campaign consisted of gathering and destroying all host fruits in Honolulu. During its investigations of the fruit fly from 1912 to 1914 the Bureau of Entomology tried extensive spraying experiments with poisoned sprays, endeavoring to kill the adult flies. Both of these methods of control failed because of the great abundance and variety of host fruits, there being over 70 varieties in Honolulu alone, some of which are bearing at all seasons of the year.

During the time these experiments were being made the Board of Agriculture and Forestry of the Territory of Hawaii engaged Prof. F. Silvestri, an Italian entomologist, to travel in Africa and Australia in search of fruit-fly parasites. In May, 1913, he arrived in Honolulu with a few living specimens of the opiine larval parasites *Opius humilis* Silvestri from Africa and *Diachasma tryoni* Cameron from Australia. In 1914 the Territorial government sent D. T. Fullaway and J. C. Bridwell to Africa to search for additional parasites. As a result of this expedition two larval parasites, an opiine (*Diachasma fullawayi* Silvestri) and a chalcid (*Tetrastichus giffardianus* Silvestri) were introduced in October of that year. All four of these parasites soon became established and were distributed to all the larger islands of the Hawaiian group.

The Bureau of Entomology, during its studies of the Mediterranean fruit fly in Hawaii and in conjunction with its quarantine work, has had an exceptional opportunity to observe the results achieved by these parasites since their introduction. A series of papers has been published,[2] giving yearly records of the work done by them, as individual

[1] Accepted for publication July 11, 1921.

[2] BACK, E. A., and PEMBERTON, C. E. PARASITISM AMONG THE LARVÆ OF THE MEDITERRANEAN FRUIT FLY IN HAWAII DURING 1914. *In* Bien. Rpt. Bd. Comrs. Agr. and Forestry Hawaii, 1913-14, p. 153-161. 1915.

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species and collectively, and the extent of infestation of different fruits by *Ceratitis capitata*. The present paper is a continuation of these records for the years 1919 and 1920.

TABLE I.—*Extent of infestation of host fruits by larvæ of Ceratitis capitata in Hawaii during 1919 and 1920*

Host fruit.	Number of fruits collected.		Number of *C. capitata* larvæ emerging.		Average number of larvæ per fruit.	
	1919	1920	1919	1920	1919	1920
Indian almond (*Terminalia catappa*)	35, 716	34, 066	300, 391	187, 811	8. 4	5. 5
Mango (*Mangifera indica*)	1, 595	1, 787	5, 857	6, 212	3. 7	3. 5
Coffee (*Coffea arabica*)	16, 055	4, 080	6, 985	2, 441	. 4	. 6
Strawberry guava (*Psidium cattleianum*)	20, 539	22, 133	25, 266	25, 662	1. 2	1. 2
Black myrobalan (*Terminalia chebula*)	8, 199	3, 373	38, 359	23, 199	4. 7	6. 9
Peach (*Amygdalus persica*)	385	10	5, 226	156	13. 6	15. 6
Satin-leaf (*Chrysophyllum olivaeforme*)	229	801	1, 136	3, 748	5. 0	4. 7
Rose-apple (*Eugenia jambos*)	....	4, 225	....	49, 329	......	11. 5
French cherry (*Eugenia uniflora*)	8, 671	5, 725	7, 643	8, 046	. 9	1. 4
West Indian medlar (*Mimusops elengi*)	....	2, 287	....	834	......	. 4
Kamani (*Calophyllum inophyllum*)	450	438	2, 682	2, 147	6. 0	4. 9
Yellow oleander (*Thevetia neriifolia*)	1, 479	2, 367	2, 462	4, 590	1. 7	1. 9
Carambola (*Averrhoa carambola*)	153	106	7	22	. 05	. 2
Chinese orange (*Citrus* sp.)	21, 804	40, 260	53, 870	94, 614	2. 5	2. 4
Guava (*Psidium guajava*)	6, 675	4, 051	65, 732	29, 168	9. 8	7. 2
Loquat (*Eriobotrya japonica*)	1, 690	....	5, 827	....	3. 4	......
Noronhia emarginata	....	194	....	28	......	. 1
Orange (*Citrus aurantium*)	216	611	710	3, 649	3. 3	6. 0
Waiawi (*Psidium guajava pyriferum*)	....	947	....	581	......	. 6
Lime (*Citrus medica limetta*)	....	154	....	182	......	1. 2
Tangerine (*Citrus nobilis*)	....	869	....	768	......	. 9

Table I gives data which show the average infestation per fruit of 21 different varieties collected about Honolulu in 1919 and 1920 and indicates in a general way the abundance of adults of *Ceratitis capitata* for those years. A comparison of this table with Table I in the records of parasitism for 1918,[3] would indicate a reduction of this pest during the past three years. For the year 1919 the average infestation of 9 varieties of host fruits was less than during 1918, and greater in 5. For the year 1920 it was less in 9 varieties, the same in 1, and greater in 6 than in 1918. It is encouraging to see that many of these reductions in infestation occurred in the most preferred hosts of the fly, notably the peach (*Amygdalus persica*) and Indian almond (*Terminalia catappa*). The peach has always been the most heavily infested fruit in Hawaii, and the average infestation for 1919 was less than for any year since the introduction of parasites, and over 33 per cent less than in 1918. The Indian almond can be found in all sections of Honolulu and is much preferred as a breeding place by the fly. It bears prolifically, and its infested fruits can be

[3] WILLARD, H. F. OP. CIT.

secured during almost every month of the year. Consequently, Indian almond has been used more than any other fruit in securing parasitism records of the fruit fly. Average infestation records of this fruit alone, of which about 30,000 are collected yearly, are a good guide to the abundance of *C. capitata* in this locality. In 1919 and 1920 this average decreased 15 per cent and 44 per cent, respectively, over that of 1918. These are the first important decreases in infestation of preferred host fruits that have taken place since parasitism records were started.

Table II, which records the parasitism of the larvæ in each host fruit by the month, reveals interesting information relative to *Diachasma fullawayi* and *Tetrastichus giffardianus*. Prior to 1920 the former had a tendency to attack its host freely in only a few fruits, namely, strawberry guava (*Psidium cattleianum*), coffee (*Coffea arabica*), French cherry (*Eugenia uniflora*), and yellow oleander (*Thevetia neriifolia*). Other fruits occasionally yielded larvæ that were parasitized by *D. fullawayi;* but the 1920 records show larvæ, in nearly all fruits under observation, to be freely attacked, especially during the latter part of the year. It caused the death of 12.1 per cent of all larvæ during the year (Table IV), which is more than double its percentage of parasitism during any of the previous five years, with the exception of 1917, when it was 7.3. *T. giffardianus* has also shown an increase over previous years, although not so great an increase as *D. fullawayi*. It has proved its value by its ability to attack its host in fleshy fruits, where the fruit-fly maggots are protected to a considerable extent from the opiine parasites. *T. giffardianus* attacks its prey within the fruit, after entering through a crack or other opening, by attaching itself to the larva while ovipositing. In this manner it can reach many larvæ which are out of reach of the opiine parasites, which oviposit only in larvæ near the surface, by piercing the skin and pulp of the fruit with their ovipositors. If fruits with thin skin and shallow pulp, like the Indian almond and coffee, were the only ones grown in Hawaii, the opiine parasites now there would probably control the fruit fly; but the fleshy fruits, such as the guava (*P. guajava*), of which there are thousands of acres and in which these parasites work with difficulty, serve as a constant source of supply of adult fruit flies. It is interesting to note that *T. giffardianus* attacked the larvæ in guava very freely during 1920. In six out of the nine months during which records were obtained it destroyed more larvæ in this fruit than the other three parasites combined. The records for 1919 and 1920 have greatly enhanced the value of this parasite.

TABLE II.—*Percentage of parasitism of larvæ of Ceratitis capitata in Hawaii in 1919 and 1920*

Host fruit.	Month	Number of larvæ emerging during first 2 to 6 days.		Percentage of parasitism.									
				Opius humilis.		*Diachasma tryoni.*		*Diachasma fullawayi.*		*Tetrastichus giffardianus.*		Total.	
		1919	1920	1919	1920	1919	1920	1919	1920	1919	1920	1919	1920
Indian almond	Jan	1,432	309	9.9	12.6	9.8	19.7	......	......	1.4	9.1	21.1	41.4
	Feb	3,657	255	28.2	14.9	12.2	.4	......	......	3.2	4.7	43.6	20.0
	Mar	197	142	36.5	23.9	13.7	2.1	2.0	0.7	4.1	2.1	56.3	28.8
	Apr	122	1,100	18.9	16.2	13.1	18.4	......	5.6	3.3	3.4	35.3	43.6
	May	......	6,841	......	26.7	......	31.6	......	.4	......	3.1	......	61.8
	June	423	240	2.6	50.0	26.2	23.8	......	......	11.3	2.9	40.1	76.7
	July	7,283	2,771	26.2	5.8	32.1	16.3	......	15.0	7.6	8.1	65.9	45.2
	Aug	7,212	4,406	7.9	9.5	8.4	13.4	......	24.1	14.9	11.6	31.2	58.6
	Sept	7,654	6,169	5.7	5.2	16.1	29.3	2.9	19.4	8.6	21.5	33.3	75.4
	Oct	8,637	3,993	4.2	2.4	13.2	56.4	1.4	6.7	6.4	6.0	25.2	71.5
	Nov	7,200	729	8.0	2.9	29.7	49.1	.2	16.2	8.2	15.0	46.1	83.2
	Dec	4,503	60	13.3	1.7	43.6	11.7	......	20.0	8.9	33.3	65.8	66.7
Mango	Apr	222	143	2.7	2.1	12.2	......	.9	......	......	......	15.8	2.1
	May	572	312	4.0	.7	16.3	7.1	.9	2.9	4.0	1.0	25.2	11.7
	June	284	461	3.6	2.6	15.8	12.6	1.1	6.3	4.2	1.5	24.7	23.0
	July	167	......	.6	......	9.6	......	1.2	......	6.0	......	17.4	......
Strawberry guava.	Jan	......	33	......	27.3	......	......	......	12.1	......	15.2	......	54.6
	Feb	......	107	......	49.5	......	......	......	9.3	......	2.8	......	61.6
	Mar	12	443	16.6	55.5	......	5.2	......	3.1	......	1.6	16.6	65.4
	Apr	152	......	48.0	......	......	......	......	......	.7	......	48.7	......
	May	2,453	509	2.0	1.2	53.4	6.5	1.5	9.4	2.1	3.9	59.0	21.0
	June	2,140	......	6.8	......	51.5	......	1.8	......	3.8	......	63.9	......
	July	......	1,786	......	7.1	......	43.0	......	17.5	......	7.2	......	74.8
	Aug	......	146	......	4.1	......	51.4	......	15.8	......	2.8	......	74.1
	Sept	......	495	......	3.2	......	9.1	......	66.3	......	5.9	......	84.5
Coffee	Jan	782	984	2.0	......	34.5	3.6	7.4	35.3	.1	......	44.0	38.9
	Feb	279	......	2.2	......	33.0	......	30.5	......	......	......	65.7	......
	Mar	380	......	.3	......	21.8	......	2.6	......	......	......	24.7	......
	Apr	9	......	33.3	......	......	......	22.2	......	......	......	55.5	......
	May	10	......	......	......	......	......	......	......	......	......	.0	......
	July	155	......	5.8	......	26.5	......	......	......	......	......	32.3	......
	Aug	......	124	......	.8	......	2.4	......	91.9	......	......	......	95.1
Black myrobalan	Apr	......	720	......	.6	......	.4	......	......	......	.1	......	1.1
	Aug	......	153	......	3.3	......	......	......	9.1	......	2.6	......	15.0
	Sept	1,337	......	4.4	......	.4	......	1.3	......	3.9	......	10.0	......
	Oct	1,478	73	14.9	......	4.6	4.1	4.9	8.2	5.8	......	30.2	12.3
	Nov	3,246	313	5.3	......	3.9	5.1	5.0	21.1	9.3	5.8	23.5	32.0
	Dec	311	328	16.1	......	1.0	7.0	1.6	23.2	47.0	15.9	65.7	46.1
Peach	Mar	89	......	.0	......	3.4	......	.0	......	39.3	......	42.7	......
	Apr	908	59	1.0	13.6	13.7	1.7	.0	.0	15.6	3.4	30.3	18.7
Satin leaf	Jan	......	164	......	28.7	......	3.7	......	4.9	......	......	......	37.3
	Feb	32	......	34.4	......	......	......	......	......	......	......	34.4	......
	Mar	115	199	67.0	30.1	......	4.0	......	34.2	1.7	1.0	68.7	69.3
Rose apple	May	......	1,933	......	1.9	......	20.2	......	1.3	......	.3	......	23.7
	June	......	8,627	......	7.3	......	24.2	......	13.0	......	1.3	......	45.8
	July	......	841	......	.7	......	54.8	......	21.8	......	5.6	......	82.9
	Aug	......	96	......	......	......	21.9	......	13.5	......	5.2	......	40.6
French cherry	Jan	205	......	2.9	......	1.5	......	......	......	.5	......	4.9	......
	Feb	501	......	11.4	......	2.8	......	.2	......	1.2	......	15.6	......
	Mar	17	......	52.9	......	......	......	......	......	......	......	52.9	......
	Apr	19	1,142	68.4	26.6	......	.3	......	1.8	......	.2	68.4	28.9
	May	6	236	50.0	28.8	16.7	8.5	......	5.1	......	.4	66.7	42.8
	June	......	1,052	......	1.8	......	39.0	......	34.1	......	.1	......	75.0
	July	......	35	......	......	......	2.9	......	77.1	......	2.9	......	82.9
	Sept	108	......	12.0	......	34.3	......	14.8	......	2.8	......	63.9	......
	Oct	322	......	35.0	......	11.8	......	18.0	......	.6	......	65.4	......
West Indian medlar.	Mar	......	92	......	1.1	......	......	......	......	......	1.1	......	2.2
	May	......	17	......	5.9	......	......	......	......	......	......	......	5.9
	June	......	29	......	......	......	......	......	......	......	......	......	......
Kamani	Jan	......	312	......	......	......	......	......	.3	......	......	......	.3
	Dec	578	......	.3	......	......	......	.2	......	......	......	.5	......
Yellow oleander	Jan	......	80	......	2.5	......	1.2	......	10.0	......	43.8	......	57.5
	Feb	......	299	......	3.0	......	1.0	......	13.4	......	13.0	......	30.4
	Mar	......	153	......	......	......	......	......	26.8	......	39.9	......	66.7
	Apr	7	35	......	11.4	......	......	......	2.9	......	57.1	......	71.4
	July	364	......	.5	......	3.0	......	20.9	......	7.7	......	32.1	......
	Aug	101	......	......	......	1.0	......	11.9	......	11.9	......	24.8	......
	Sept	12	......	......	......	......	......	......	......	......	......	......	......
	Oct	25	35	4.0	......	16.0	......	36.0	62.9	8.0	11.4	64.0	74.3
	Dec	22	......	13.6	......	......	......	22.7	......	9.1	......	45.4	......
Carambola	Sept	6	......	......	......	16.7	......	16.7	......	......	......	33.4	......

TABLE II.—*Percentage of parasitism of larvæ of Ceratitis capitata in Hawaii in 1919 and 1920*—Continued

Host fruit.	Month.	Number of larvæ emerging during first 2 to 6 days.		Percentage of parasitism.									
				Opius humilis.		*Diachasma tryoni.*		*Diachasma fullawayi.*		*Tetrastichus giffardianus.*		Total.	
		1919	1920	1919	1920	1919	1920	1919	1920	1919	1920	1919	1920
Chinese orange..	Jan...	......	382	......	7.9	......	1.8	......	0.8	......	0.5	......	11.0
	Feb...	705	292	0.6	3.1	10.6	.7	1.0	1.7	1.8	1.0	14.0	6.5
	Mar...	440	129	1.6	20.9	6.6	.8	1.4	1.6	......	3.1	9.6	26.4
	Apr...	735	292	2.0	16.8	7.9	2.4	.1	1.7	.8	2.7	10.8	23.6
	May...	350	207	1.4	4.3	11.4	12.1	......	3.4	......	11.1	12.8	30.9
	June..	304	103	.3	14.6	6.9	......	......	......	5.2	1.0	12.4	15.6
	July...	60	279	......	4.7	10.0	10.4	3.3	4.3	10.0	3.6	23.3	23.0
	Aug...	......	159	......	8.2	......	4.4	......	.6	......	7.6	......	20.8
	Sept..	61	......	3.3	......	3.3	......	3.3	......	......	......	9.9	......
	Oct...	53	84	3.8	3.6	......	1.2	......	3.6	......	20.2	3.8	28.6
	Nov...	244	368	2.0	2.2	.8	9.5	1.6	20.1	3.3	5.4	7.7	37.2
	Dec...	245	147	4.5	2.7	1.2	4.8	4.1	4.8	2.0	12.2	11.8	24.5
Guava..........	Jan...	......	1,258	......	8.7	......	5.9	......	5.2	......	7.9	......	27.7
	Feb...	140	1,139	2.9	10.0	1.4	13.8	1.4	3.2	......	24.7	5.7	51.7
	Mar...	1,233	151	1.9	11.3	4.0	2.0	.5	4.0	1.0	49.7	7.4	67.0
	Apr...	679	40	......	5.0	23.3	......	.3	5.0	11.5	55.0	35.1	65.0
	May...	1,115	......	.2	......	27.5	......	.6	......	13.8	......	42.1	......
	June..	949	......	4.5	......	13.4	......	1.1	......	12.5	......	31.5	......
	July...	580	251	2.4	2.0	22.4	4.8	.7	2.0	18.6	21.9	44.1	30.7
	Aug...	292	......	.3	......	7.9	......	21.6	......	31.8	......	61.6	......
	Sept..	141	184	......	1.6	8.5	4.9	14.9	16.3	8.5	22.3	31.9	45.1
	Oct...	93	919	......	.5	2.2	14.8	14.0	14.8	9.7	10.9	25.9	41.0
	Nov...	90	338	......	......	......	14.2	......	13.0	13.3	37.0	13.3	64.2
	Dec...	237	277	8.8	......	3.8	1.8	4.6	4.3	12.2	19.1	29.4	25.2
Loquat..........	Feb...	498	......	2.8	......	11.2	......	3.6	......	.4	......	18.0	......
Noronhia........	July...	179	......	.6	......	.6	......	......	......	......	......	1.2	......
	Aug...	43	......	......	......	7.0	......	......	......	......	......	7.0	......
Orange..........	Feb...	......	21	......	4.8	......	......	......	4.8	......	4.8	......	14.4
	Mar...	21	330	......	.6	......	1.2	......	3.0	......	7.6	......	12.4
	Apr...	24	142	......	......	......	......	......	2.8	......	7.7	......	10.5
	May...	5	......	......	......	......	......	......	......	......	......	......	......
	Sept..	42	......	......	......	......	......	......	......	2.4	......	2.4	......
	Oct...	68	......	......	......	......	......	4.4	......	4.4	......	8.8	......
	Nov...	26	......	......	......	......	......	......	......	11.5	......	11.5	......
	Dec...	8	333	......	.3	......	2.4	......	1.2	......	20.4	......	24.3

Tables III and IV record the work of each parasite in all fruits collected over monthly and yearly periods. In Table III, the percentages of parasitism by *Diachasma fullawayi* for 1919 are typical of those for previous years. A comparison of those figures with percentages for 1920 in the adjacent column on the right again reveals the increase in the effectiveness of this parasite. In two months of 1920, January and August, its work exceeds that of any one of the other three parasites, and in six other months it was second in effectiveness. As indicated in a previous publication,[4] the ability of *D. tryoni* and *fullawayi* to destroy *Opius humilis* when they occur in the same host larva influences the amount of parasitism by *O. humilis* to a great extent. *O. humilis* has always been more abundant during the cooler months of the year, when the two species of Diachasma are less active and have a tendency to hibernate; and it decreases greatly in numbers during the warmer months, when it must contend with maximum numbers of Diachasma. In records of parasitism for 1916 [5] the effectiveness of *O. humilis* during five months out of the year was greater than that of both species of

[4] PEMBERTON, C. E., and WILLARD, H. F. INTERRELATIONS OF FRUIT-FLY PARASITES IN HAWAII. *In* Jour. Agr. Research, v. 12, no. 5, p. 285–296, pl. 10–13. 1918.

[5] PEMBERTON, C. E., and WILLARD, H. F. FRUIT-FLY PARASITISM IN HAWAII DURING 1916. *In* Jour. Agr. Research, v. 12, no. 2, p. 103–108. 1918.

Diachasma. In 1917 and 1918 [6] it was greater during two months; and in 1919 and 1920, for one month out of each year. The control exerted over *O. humilis* by Diachasma, reducing parasitism by the former as that of the latter increases, is clearly shown in Table IV. In 1915 *O. humilis* destroyed 31.5 per cent of all *Ceratitis capitata* larvæ under observation, or 83.1 per cent of the total parasitized larvæ. In 1920 its parasitism was only 9.4 per cent of all larvæ, and 18.1 per cent of the total parasitized larvæ. This great decrease in the numbers of *O. humilis* over a period of six years is due almost entirely to the cannibalistic habits of *D. tryoni* and *D. fullawayi*. *Tetrastichus giffardianus* probably destroys small numbers of *O. humilis*, as well as *D. tryoni* and *D. fullawayi*. Studies of the interrelations of these parasites [7] show that *T. giffardianus*, which does not resort to cannibalism, is capable of destroying opiine parasites occurring in the same host larva, probably by starvation. This parasite deposits about 10 eggs at one time in a single fruit-fly larva. The larvæ hatching from these eggs usually have no more than one opiine larva to contend with, and they absorb the food material of the host so rapidly that the opiine larva usually dies. Before death, however, the opiine larva often destroys many of the *T. giffardianus* larvæ, but no instance has been observed where the opiine larva survived, although such a case may be possible. Should *T. giffardianus* continue in numbers and effectiveness, it will doubtless cause a decrease in the numbers of the opiine parasites; and it will be interesting to note what effect the new proportions of parasitism will have on the amount of infestation by *C. capitata*.

The records of fruit-fly parasitism for 1919 and 1920 have shown several interesting facts in connection with the efforts to control the Mediterranean fruit fly by introduced parasites. The continued activities of these parasites during the past six or seven years, and the fact that they have destroyed approximately 50 per cent of the fruit flies developing during the past four years, have caused a noticeable decrease in the infestation of some of the most preferred host fruits of the fly. A great check has been exerted on the activities of *Opius humilis* by the two species of Diachasma until, in 1920, it was the least effective of the three opiines and parasitized a smaller percentage of larvæ than during any year since its introduction. *Diachasma fullawayi* and *Tetrastichus giffardianus* have increased greatly in value, and have proved their ability to attack fruit-fly larvæ in almost any fruit. While the use of parasites does not control the Mediterranean fruit fly in Hawaii, it has met with a large degree of success, as compared with other methods of combating this pest, and has decreased the infestation of many edible fruits to a marked extent.

[6] PEMBERTON, C. E., and WILLARD, H. F. WORK AND PARASITISM OF THE MEDITERRANEAN FRUIT FLY IN HAWAII DURING 1917. *In* Jour. Agr. Research, v. 14, no. 13, p. 605–610. 1918.
WILLARD, H. F. WORK AND PARASITISM OF THE MEDITERRANEAN FRUIT FLY IN HAWAII DURING 1918. *In* Jour. Agr. Research, v. 18, no. 8, p. 441–446. 1920. Literature cited, p. 446.

[7] PEMBERTON, C. E., and WILLARD, H. F.. INTERRELATIONS OF FRUIT-FLY PARASITES IN HAWAII. *In* Jour. Agr. Research, v. 12, no. 5, p. 285–296, pl. 10–13. 1918.

TABLE III.—*Total parasitism of all larvæ of Ceratitis capitata collected in Hawaii during 1919 and 1920 (monthly averages)*

Month.	Number of larvæ.		Percentage of parasitism.									
			Opius humilis.		*Diachasma tryoni.*		*Diachasma fullawayi.*		*Tetrastichus giffardianus.*		Total.	
	1919	1920	1919	1920	1919	1920	1919	1920	1919	1920	1919	1920
January	2,419	3,522	6.8	6.7	17.1	5.2	2.4	12.4	0.9	4.8	27.2	29.1
February	5,812	2,113	19.4	10.6	11.8	7.7	1.9	4.4	2.4	16.0	35.5	38.7
March	2,504	1,639	7.7	23.6	7.6	2.7	1.0	8.7	2.2	10.9	18.5	45.9
April	2,877	3,673	4.9	15.0	13.3	5.9	.2	2.6	8.0	2.8	26.4	26.3
May	4,511	10,055	1.8	19.4	38.8	26.4	1.1	1.3	5.1	2.7	46.8	49.8
June	4,100	10,512	5.1	7.6	34.3	24.8	1.3	14.4	6.2	1.2	46.9	48.0
July	8,788	5,963	22.0	5.2	28.9	28.9	1.0	16.0	8.1	7.8	60.0	57.9
August	7,648	5,084	7.4	8.7	8.3	13.7	1.0	24.1	15.5	10.5	32.2	57.0
September	9,361	6,848	5.4	4.8	13.8	27.2	3.0	22.7	7.9	20.4	30.1	75.1
October	10,676	5,104	6.5	2.0	11.7	46.8	2.6	8.5	6.1	7.1	26.9	64.4
November	10,806	1,748	6.9	1.7	21.1	26.1	1.6	17.3	8.4	15.6	38.0	60.7
December	5,904	1,145	11.6	.5	33.5	4.4	.6	9.7	9.9	18.4	55.6	33.0

TABLE IV.—*Total parasitism of all larvæ of Ceratitis capitata collected in Hawaii from 1915 to 1920 (yearly averages)*

Year.	Number of larvæ.	Percentage of parasitism.				
		Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1915	28,010	31.5	0.3	5.9	0.2	37.9
1916	83,304	17.2	13.3	2.1	.6	33.2
1917	72,139	12.7	20.3	7.3	7.2	47.5
1918	63,480	12.4	34.6	2.6	6.2	55.8
1919	75,406	9.4	19.6	1.6	7.6	38.2
1920	57,406	9.4	22.7	12.1	7.7	51.9

ACID PRODUCTION BY RHIZOPUS TRITICI IN DECAYING SWEET POTATOES[1]

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INTRODUCTION

Weimer and Harter[2] have recently shown that the carbohydrate losses from sweet potatoes undergoing decay by *Rhizopus tritici* Saito. exceed the weight of carbon dioxid evolved, and that the difference is considerably greater than the probable amount utilized in the construction of fungous tissue. They report an increase in the hydrogen-ion concentration of the juices of the decayed material, and indicate the probability of alcohol formation. They conclude:

> That acids are produced in considerable abundance seems quite evident, and that alcohol is formed seems probable. The carbohydrates required for the manufacture of acids and alcohol together with that utilized directly in the production of fungous material will probably account to a large extent for the decrease in the sugars and starch which are not accounted for by the CO_2 evolved.

In the hope of securing additional information regarding the carbohydrate changes involved, particularly the identity of the acid or acids produced, the present writer, working with material courteously supplied by Weimer and Harter, has carried on certain additional experiments. The results, which are here presented, are confirmatory and show that the principal products of the fermentation are ethyl alcohol and acetic acid, but that formic acid, a trace of butyric acid, acetone, an unidentified aldehyde, and traces of at least two nonvolatile acids, one of which is lactic and the other probably succinic, are produced, and that a small amount of ammonia appears among the nitrogenous decomposition products.

EXPERIMENTAL WORK

The decayed sweet potatoes employed resulted in most instances from artificial inoculation with *R. tritici*, but in a few cases the decay resulted from natural infection. Some tests were made in the early stages of decay while it was incomplete but actively progressing. In these cases only the softened portions of the tubers were employed. Most of the tests were made on material which had undergone complete decay in moist chambers in the incubator at 30° C. after which they were held for a period which varied from 1 or 2 to 18 or 20 days.

The results were substantially identical in all cases. For each trial about 10 tubers were placed in cotton cloth and subjected to heavy pres-

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[1] Accepted for publication Mar. 15, 1923.

[2] Weimer, J. L., and Harter, L. L. respiration and carbohydrate changes produced in sweet potatoes by rhizopus tritici. *In* Jour. Agr. Research, v. 21, p. 627-635. 1921. Literature cited, 634-635.

sure in a meat press. About 1100 cc. of brown liquid, acid to litmus, was thus secured. It was rendered alkaline with sodium carbonate and distilled with steam under approximately constant volume. After 100 to 150 cc. of distillate (which began to come over at about 92° C.) had been collected, distillation was interrupted and the material allowed to partially cool. An excess of phosphoric acid was then added and the copious precipitate of organic matter which appeared was centrifuged off, and the filtrate returned to the apparatus and distilled with steam at constant volume until two or three liters of distillate had been collected and the condensing vapors no longer gave an acid reaction to litmus. Distillation was then continued under diminishing volume until only 150 or 200 cc. remained in the distilling flask. This residue was allowed to cool and extracted with ether for several hours for the recovery of non-volatile organic acids.

The distillate obtained from the alkaline liquid possessed a neutral reaction and a strong odor of ethyl alcohol. It was inflammable, burning with a blue flame. It was found that 5 cc. yielded a very copious precipitate of iodoform when subjected to the usual potassium hydroxid iodin test. Heated with a few drops each of sulphuric and acetic acids, it yielded distinctly the odor of ethyl acetate.

With ammoniacal silver nitrate, 5 cc. of the distillate gave a heavy silver mirror. It also restored the color to magenta solution decolorized with sulphurous acid. It developed a pungent but not a lemon odor and a yellow color on boiling with sodium hydroxid. The color was at first clear yellow, then slighty cloudy, becoming clear again on further boiling, with the final development of reddish-brown, but not the yellow-orange color or the odors characteristic of either acetic or propionic aldehyde. The resorcine sulphuric acid tests for formaldehyde yielded a brown ring and a white precipitate, soon turning brown. The red color characteristic of formaldehyde did not appear. The gallic acid test with sulphuric acid was also negative for formic aldehyde.

The liquid gave a slight but distinct positive test for acetone with the Gunning iodoform test. A portion of the distillate, slowly heated with a distinct excess of Fehling's solution in a distilling flask and then distilled, yielded reduced copper in the flask and a distillate which reacted positively for alcohol but no longer gave the aldehyde or acetone reactions. The original distillate gave a positive reaction for ammonia with Nessler's reagent, and it was noted also in performing the Gunning acetone test that a black precipitate (nitrogen iodid), which disappeared on standing, formed immediately on the addition of iodin and before ammonia was used.

These tests show that the distillate contained a high percentage of ethyl alcohol, appreciable amounts of an unidentified aldehyde (not formic and probably not acetic or propionic), traces of acetone, and small amounts of ammonia.

The entire distillate from phosphoric acid was titrated with standard barium hydroxid and evaporated to dryness on a steam bath. It usually contained from 30 to 40 cc. of normal acid, though some of the freshly decaying samples yielded only 10 or 12 cc. The dried barium salts were extracted with 10 to 20 volumes of absolute alcohol for several hours with frequent trituration, filtered and washed with alcohol. The filtrate when evaporated yielded only a very small residue. When warmed with a drop or two of sulphuric acid the residue gave a rancid odor. The addition of a few drops of ethyl alcohol and further heating developed

an agreeable odor suggestive of pineapple, indicating the presence of butyric acid. The barium salts that were insoluble in alcohol were taken up in water, filtered free from the small amount of insoluble carbonates and decomposed by an excess of sulphuric acid. The barium sulphate was removed by filtration and the volatile acids removed by distillation from the filtrate. Tests for the identification of the acids were made on this distillate or on the sodium or barium salts obtained from it.

The reaction with ferric chlorid was positive for acetates and formates. With silver nitrate, a white crystalline precipitate, soluble in ammonia, was produced. On standing, or more promptly on heating, the precipitate acquired a dark tint, but the separation of metallic silver causing it was always small. Mercurous nitrate produced a white crystalline precipitate which also took on a grayish tint on standing or on heating. Mercuric chlorid gave a white precipitate of mercurous chlorid on heating, but its volume was very small for the amount of salt tested. Heated with sulphuric acid the salts gave off the odor of acetic and formic acid. Slight effervescence accompanied the reaction, yielding a gas which in carefully carried out tests could be ignited, burning for an instant with a blue flame. The ester obtained on heating the salt with sulphuric acid and alcohol suggested both methyl and ethyl acetates, in comparison with parallel tests on pure known salts alone and combined. The free acids were warmed at 45° C. with an excess of mercuric oxid, filtered, and the filtrate heated to boiling; a slight but distinct precipitate of metallic mercury was thrown down. White crystals of acetate of mercury appeared in the liquid on cooling. The sodium salt heated with para-toluidin and hydrochloric acid yielded an acid toluid which when purified and twice recrystallized melted at 145°–146° C., uncorrected. Acet-p-toluid melts at 148.2°C., corrected. A portion of the barium salts was purified by twice redistilling from strong sulphuric acid and again obtained as the barium salt. After recrystallization the barium content of the dehydrated product determined gravimetrically by precipitation with sulphuric acid was 53.53 per cent. A synthetic sample prepared from pure glacial acetic acid and the barium hydrate used in the work also yielded an average of 53.53 per cent of barium. The theoretical figure for pure barium acetate is 53.79 per cent barium.

From the foregoing tests it is evident that the volatile acid obtained was chiefly acetic but that a small amount of formic and a trace of butyric were also recovered.

The ether extract was examined for nonvolatile acids. On standing fine needle crystals appeared in the sirupy matrix remaining after evaporation of the ether. Water was added and the resulting solution titrated against phenolphthalein with standard barium hydrate, 50 to 60 cc. of $N/10$ solution being required. The resulting barium salts were evaporated to dryness, triturated with 10 to 12 cc. of water, filtered, washed with a few cubic centimeters of water, and the filtrate and wash water treated with strong alcohol till the concentration was 90 per cent by volume. It was then placed in the ice box for a day or two, after which the precipitate was filtered off and washed with alcohol.

The filtrate was evaporated to a small volume, diluted with 20 cc. of water and carefully treated with $N/10$ sulphuric acid, drop by drop, as long as calcium sulphate precipitated. The excess sulphuric acid added was removed by one or two drops of barium hydrate solution and the barium sulphate removed by filtration and washed. The filtrate and washings were evaporated on the water bath to a small

volume and tested for lactic acid by the Kelling ferric chlorid test and by Uffelmann's test, both of which gave positive reactions. The remainder of the liquid was placed in a test tube fitted with a conducting tube leading to a second test tube containing 1 cc. of water. On heating the material in the first tube it decomposed, giving off white vapors, which were absorbed by the water in the second tube. This material when boiled with 5 cc. of 10 per cent sodium hydroxid became first clear-yellow, then turbid, opaque and yellow-orange, giving a penetrating characteristic odor, thus confirming the previous tests for lactic acid.

The residue of barium salts of nonvolatile acids remaining undissolved after trituration with the 10 to 12 cc. of water, as indicated in the preceding paragraph, was dissolved in 50 cc. of hot water and decomposed with sulphuric acid. Any trace of excess acid was avoided. The material was concentrated on the steam bath to 10 cc., filtered into a weighed test tube, and evaporated to dryness on a steam bath while removing the vapors by an air current through a piece of glass tubing inserted in the neck. The dried residue of about .05 gm. was heated for 30 minutes with .3 gm. of p-toluidin in a bath at 210° C., employing a reflux air cooler. After cooling, 5 cc. of 50 per cent alcohol were added, and then it was boiled, thoroughly cooled, and filtered. The crystals obtained were dissolved in 5 cc. of alcohol and recrystallized on a watch glass. White needle crystals, presumably succintoluid, separated out, but the yield was almost microscopic in volume and too impure, as shown under the lens, to employ in a melting point determination. The precipitate filtered from the 90 per cent alcohol solution was also tested as outlined above for succinic acid but without positive results. The evidence obtained therefore indicates the presence of a trace of lactic acid and perhaps also succinic acid.

In order to make sure that the products identified were not the result of the activities of bacteria or other secondary organisms following the fungus, the work was repeated with substantially identical results, employing both sweet potato broth and sterilized sweet potatoes in flasks, as well as raw blocks of sweet potato cut from the tubers under aseptic conditions and placed in sterilized flasks. Raw sweet potato juice secured by grinding sound tubers of the same variety as those used in the inoculation experiments and subjecting the pulp to pressure in the meat press yielded a neutral distillate free from ammonia.

SUMMARY

It may be concluded that the fermentation produced in sweet potatoes decaying through the action of *Rhizopus tritici* is of the familiar alcohol-acetic acid type, in which, in addition to alcohol and acetic acid, much smaller amounts of formic, butyric, lactic, and succinic acids are found, as well as acetone and an unidentified aldehyde, and that ammonia is among the nitrogenous decomposition products.

TEMPERATURE EFFECTS IN PLANT METABOLISM[1]

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INTRODUCTION

In recent years considerable attention has been given by ecologists to climatic factors as determining plant distribution. Among the more recent publications in this field is the work of Livingston and Shreve (*27*).[2] The extensive observations of Bonnier (*1*, *2*) upon anatomical and physiological modifications in plants of the same species, grown at different altitudes, are also noteworthy in this connection. This investigator has recently detected a complete change of variety in plants subjected for several years to such change of environment. To the physiologist, adaptations of this sort are explainable by the assumption that changes in the intensity of the various climatic factors disturb the chemical and physical equilibria which direct the growth process.

Followers of agricultural science are familiar with general relations between variations of climate and differences in the chemical composition of plants. Thus Hall (*9, p. 83*) states:

> Even on the Rathamsted plots, where the differences in the supply of nutrients are extreme and have been accumulating for 50 years, the composition of the grain changes more from one season to another than it does in passing from plot to plot.

Beginning with the work of Richardson (*31, p. 67; 32, p. 25*) on analyses of grains from various regions of the United States considerable work has been done in this country upon the problem of environmental effects in the chemical composition of plants. Le Clerc (*18*, *19*) has shown that the hot arid climate of Kansas is conducive to high protein content of wheat grain, irrespective of the types of soil tested by him. Richardson found no difference in composition of maize from different regions. No decided correlation between climatic factors and the composition of sweetcorn was found by Straughn and Church (*38*) in an investigation confined to the Atlantic Coast States. On the other hand, Wiley (*41*) found a distinct correlation between the sugar content of the sugar beet and the latitude of the State experiment stations which cooperated in his investigation. He concluded that temperature was the effective climatic factor in this case.

Apparently there exists an open question as to whether such climatic influences as have been mentioned here operate only upon the plant or act also indirectly through modification of the composition of the soil. Thus, while Lawes and Gilbert (*17*) found the proportion of grain in the wheat crop of Great Britain decreased by excessive rainfall, they attributed the effect partly to loss of nitrates from the soil by leaching. Furthermore, Gericke (7, *8*) has shown that the protein content of wheat

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[2] Reference is made by number (italic) to "Literature cited," p. 28–30.

grain can be increased by adding nitrates to the soil at a late stage of growth. He attributes the characteristically low protein content of wheat of the Pacific Coast States to deficiency of soil nitrates rather than to purely climatic influences. Finally, Lipman and Waynick (*20*) have described rather profound effects of climate upon biological and chemical properties of the soil.

Recent investigations in this field have included attempts either to analyze climatic conditions more closely in relation to growth out of doors, or to control some of the climatic factors within greenhouses. Thus, Briggs, Kidd, and West (*4*) analyzing the data of Kreusler for the growth of maize, found the increase of weight per unit of leaf area better correlated with variations of atmospheric temperature than with variations of either illumination or rainfall. Their methods of treatment have been unfavorably criticised, however, by Fisher (*5*). In the case of peas grown in water cultures Brenchley (*3*) found the percentage rate of increase of the dry matter correlated with the temperature of the greenhouse at the foreperiod of growth, but with both temperature and sunshine thereafter. Walster (*40*) conducted sand cultures of barley in greenhouses controlled approximately to 15° and 20° C., but with the atmospheric humidity subject to influence by temperature changes. With a liberal supply of nitrates provided in the nutrient salts the plants grown at the higher temperature were excessively vegetative, while the other cultures supported normal culm formation. Under these conditions the leaves of the plants grown at the higher temperature were comparatively rich in soluble nitrogenous compounds, while they were relatively poor in sugars and other soluble carbohydrates. The reverse of this relation obtained with plants grown at the lower temperature. Differences were found in the distribution of various forms of phosphorus compounds in the plant tissue of the two types of cultures. The greatest quantitative difference found by chemical analysis, however, related to the polysaccharids. There were nearly 3 per cent more of these in the dry matter of the plants grown at 15° than in that produced at 20°.

The foregoing abstracts may serve to indicate the relative importance of temperature differences in climatic effects upon plant growth, as well as the apparently specific compositional response of the plant thereto.

It can be readily appreciated that such responses may bear important relations to disease resistance in the organism. As a matter of fact, plant pathologists have been giving increasing attention to environmental factors (*12*, *13*, *14*, *29*, *39*) in the investigation of disease relations. Furthermore, the work of Kraus and Kraybill (*16*) indicates important relations to fruitfulness of the ratio between nitrogen and carbohydrates of plant tissues. The possible practical importance of climatic modifications of plant composition thus becomes apparent.

EXPERIMENTATION

The more significant of the experiments to be described here were conducted in chambers especially constructed for regulation of atmospheric temperature and humidity.[3] Pending the development of these chambers the following preliminary test was made in greenhouses regulated roughly within different temperature ranges.

[3] Chambers for a similar purpose have been previously developed by Hottes, as mentioned by Peltier (*2?, p. 41?*), later developments have been described by Johnson (*12*).

RED CLOVER (TRIFOLIUM PRAETENSE) IN SOIL CULTURES WITHIN GREENHOUSES

The soil employed was Miami silt loam as described in a previous publication (*10, p. 237*). It was compacted moderately in glazed stoneware jars 21 cm. in diameter and 13 cm. deep (1-gallon crocks). These held conveniently 5 kgm. each of the air-dried soil. Two gm. of $CaCO_3$ were mixed with each portion of soil. While filling the jars two of the cylindrical form of auto-irrigator (unground atmometer cups) introduced by Livingston (*21, 11*) were placed in the soil. These were connected with water reservoirs which could be adjusted vertically to regulate the plane of water in the soil of each jar separately.

On January 2, 1918, when the soil masses had attained a moisture content of 14 per cent (by weight), seeds from a vigorous commercial stock were sown in four jars. The jars remained in a greenhouse with a temperature range of 15.5° to 21° C. until the seedlings appeared, a period of six days.[4] Two of the jars were now transferred to another greenhouse with a temperature range of 10° to 15.5° C. The two pairs of cultures were placed in the same relative position in the southwest corner of the two greenhouses. A thermometer was plunged near the center of the soil mass in one pot of each pair and another was suspended with its bulb about 15 cm. above and midway between the two jars. The readings of these instruments were recorded daily, usually at about 4.30 p. m.

By occasional adjustment of the height of the water column connected with the irrigators the moisture contents of the soil in the several jars were increased and equalized. On January 14 the plane of soil moisture was 17.8 and 19.8 per cent in the cultures of the colder house and 19 and 20.3 per cent in those of the warmer one. These values approximate 40 per cent of saturation. The optimum content of this soil for red clover under similar greenhouse conditions, but in large containers, has been found to be 50 per cent of saturation.

The plants were reduced in number by removing the poorer individuals from time to time. This process was discontinued on February 10, when 8 plants per jar remained. After the plants reached considerable size and drew moisture rapidly from the soil the latter contracted, thus breaking contact with the irrigating cups. This caused variations in the plane of soil moisture among the several cultures.[5]

During the growth period the humidity of the air and approximate degree of illumination in the two houses were compared by means of the spherical form of the white and black atmometers devised by Livingston (*22, 23, 24*). In this case the water loss from the standard white porous clay instrument is employed as an index of the moisture deficit of the atmosphere, while the added evaporation from the blackened sphere serves as a comparative measure of light intensity.

The tops of the plants were harvested when the seventh and eighth leaves were emerging from the stools. This occurred on March 29 and April 13 at the higher and lower temperature ranges, respectively. On the former date the soil moisture was 10.8 and 15.4 per cent in the cooler house and 7.8 and 9.6 per cent in the warmer one. The value in the former case decreased to 7 per cent at the time of harvesting. After

[4] In view of the results of Kidd and West (*15*) relative to physiological predetermination, it would have been preferable to rear the seedlings at the temperatures in which the plants were to be reared.

[5] An improved form of irrigator which corrects this difficulty is described by Livingston (*26*).

drying at 100° C. the separate portions of tops were ground and subjected to chemical analysis by the official methods (*42*) commonly employed. The acid-hydrolyzable material is computed as glucose from the reducing power of the extract obtained with boiling 1.25 per cent H_2SO_4 in determining crude fiber. Table I contains the data of climatic measurements and Table II shows the composition of the plants in this test.

TABLE I.—*Climatic data of the environment of clover cultures*

SAME PERIOD AS OTHER CULTURES

House temperature.	Air temperature.			Total evaporation.		Ratio of evaporation.	Soil temperature.		
	Minimum.	Maximum	Average.	White atmometer.	Black atmometer	Black to white atmometer.	Minimum.	Maximum.	Average.
	° C.	*° C.*	*° C.*	*Cc.*	*Cc.*		*° C.*	*° C.*	*° C.*
Cooler	8. 0	31. 0	14. 3	711	1, 085	1. 53	5. 0	31. 0	15. 8

FULL PERIOD OF GROWTH

House temperature.	Air temperature: Minimum.	Maximum	Average.	White atmometer.	Black atmometer	Black to white atmometer.	Soil temperature: Minimum.	Maximum.	Average.
Cooler	8. 0	32. 0	15. 0	857	1, 306	1. 52	5. 0	31. 0	17. 2
Warmer	13. 3	29. 0	20.6	1, 047	1, 420	1. 36	11. 0	32. 0	20. 2

TABLE II.—*Composition of clover tops grown in different greenhouse environments*

House temperature.		Dry matter of tissues.	Yield of dry matter.	Crude protein.	Ether extract.	Crude fiber.	Pentosans.	Polysaccharids.[1]
		Per cent.	*Gm.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Cooler	(A)	21. 5	12. 75	18. 5	5. 1	15. 0	9. 2	10. 5
	(B)	19. 8	10. 80	21. 1	6. 1	14. 2	8. 3	13. 9
Average		20. 7	11. 78	20. 0	5. 6	14. 6	8. 8	12. 2
Warmer	(A)	20. 6	12. 55	21. 7	5. 5	13. 8	9. 4	8. 0
	(B)	22. 7	9. 45	22. 5	3. 4	14. 8	9. 6	8. 6
Average		21. 7	11. 00	22. 1	4. 5	14. 3	9. 5	8. 3

[1] Hydrolysis by boiling with 1.25 per cent H_2SO_4 for 0.5 hour. Results are in equivalents of glucose.

Inspection of the climatic data shows that, with the exception of the maximal values which occurred toward the close of the experiment, a difference of about 5° C. was maintained in atmospheric temperatures. The average observed temperatures of the two houses approximated 15° and 20.6° C, for the full period of growth in each case. For the period of time when both pairs of cultures were growing simultaneously, the water loss from the white atmometer was 47 per cent greater in the warmer house than in the cooler one. This may be considered an index of the relative vapor pressure deficits of the atmosphere in the two cases. Apparently the relative humidity was nearly equal in the two greenhouses for the same relative humidity in both cases would bear the same ratio to each other as the total vapor pressures at saturation. Thus, with the latter values fixed at 12.2 mm. for 15° and 18.2 mm. at 20.5°

C. the evaporation value for any relative humidity below 100 per cent would bear the ratio 18.2:12.2 or 149:100. Hence, evaporation should be 49 per cent greater at the higher temperature than at the lower one. In the case of the black atmometers, which correspond more closely to plants than do the white ones as regards water loss when illuminated, it appears that the tendency to transpiration was about 31 per cent greater in the warmer house than in the cooler one. As will appear subsequently, this lesser difference of the black as compared with the white instruments is probably due to greater duration of sunlight in the cooler house.

Considering the increase of evaporation from the black atmometer over that from the white one as an index of the exposure to sunlight, it appears that the latter was appreciably greater in the cooler house than in the warmer one. This difference is probably due to the fact that the former house was on the western and the latter on the eastern side of a group of four parallel ranges of greenhouses. As a result, the cooler house received direct sunlight for a greater part of the day than the warmer one. The atmometric data indicate that the solar radiation was about 44 per cent greater in the former case.

The only significant difference in chemical composition between the plants grown at the two temperature ranges resides in the acid-hydrolyzable carbohydrates or polysaccharids. These compounds are included in the group of roughly defined hemicelluloses. There is no overlapping of the values for individual cultures at the different temperatures in this case, and the average value of the reducing sugars formed is about 4 per cent greater at the lower than at the higher temperature. Thus, a combination of relatively greater illumination, lower saturation deficit of the atmosphere, and lower atmospheric temperature was accompanied by increased storage of carbohydrates in the clover plant.

BUCKWHEAT (POLYGONUM FAGOPYRUM) IN SOIL CULTURES WITHIN CLIMATIC CHAMBERS

In view of the desirability of conducting plant cultures with variation of only one climatic factor, while limiting the others to as constant values as practicable, special chambers were constructed for this purpose. These were placed in the southern end of the greenhouse previously used for the higher temperature range. A section about 8 feet long at this end of the house was separated from the main portion by a partition of artificial boarding. The southern side of this partition was painted glossy white so as to reflect light into the climatic chambers which stood close by on the ends of the usual greenhouse benches. It was necessary to exclude sunshine from the culture chambers, for the radiation effects otherwise produced were uncontrollable. For this purpose curtains of bleached muslin were suspended from the ridge to the gutters of the greenhouse compartment and on the end of the house. These were pushed aside on cloudy days.

Plate 1, A, shows the climatic chambers in operation, together with portions of the humidifiers. The general arrangement consisted of humidifying chambers in which the minimum possible temperature imparted to the conditioned air current was limited by the temperature of the water supply from Lake Mendota.

As used, the temperature of the water was raised by electric heaters with thermostatic controls. On leaving the humidifier the air passed

through a cylindrical connection to the culture chamber. In this passage its temperature was raised by electric heaters which were controlled by a thermostat placed within the latter chamber. By this treatment the relative humidity of the air was reduced toward the desired value.

HUMIDIFIERS

The humidifiers were constructed from heavy galvanized iron sheeting after the plan of one described by Shamel (*34*). At the higher temperature 50 per cent greater length and capacity were provided than at the lower one. Each consisted of an upper tray 37.5 cm. wide and 5.6 cm. deep, resting upon a chamber 30 cm. in both width and depth. The tray consisted of troughlike sections 5 cm. in width and depth, so soldered together as to provide slits through which could be passed strips of toweling 30 cm. wide and 45 cm. long. The latter which were of coarse, open-meshed linen, were secured near one end to the bottom of the humidifying chamber by means of iron rods passed through loops in the toweling. The other end was drawn firmly through the slit above and fastened with brass clips. In this way one end of the toweling was bathed by water as it flowed through the tray and the other end was immersed in the overflow as it returned over the bottom of the chamber below. Baffle plates were arranged to direct the water and air currents completely in contact with the toweling. The water was heated by luminous radiator units of either 250 or 500 watts capacity. These were placed in copper cylinders which were sealed concentrically within iron ones, the water flowing between the two. A mercury thermoregulator which controlled these heaters was immersed in the water current near the entrance to the tray. Passing to the chamber below by a drainage tube, the water escaped to the drain through a siphon, thus preventing interference with its removal by the air current. The latter was provided by a No. 00 Buffalo forge blower operated continuously by a small electric motor. Air entered the humidifier at one end through a circular orifice 9 cm. in diameter and escaped to a heating cylinder through a similar orifice near the other end of the chamber. Here it was further heated by eight small cylindrical units of a capacity of 28 watts each, operated by a bimetallic thermostat suspended on the wall of the culture chamber. In all cases the thermostats were operated on 110 volts alternating current through pony relay instruments protected by ample resistance.

Heat insulation of the humidifier was provided by a blanket formed by supporting thin asbestos sheeting in cheesecloth. With 12 towels in the installation for lower temperature, air which was passed through the humidifier from the surrounding greenhouse at a probable rate of air replacement in the culture chamber of at least once in 5 minutes acquired a relative humidity of practically 100 per cent at 12° C. In the other installation, equipped with 18 towels, the relative humidity was adjusted to about 90 per cent at 18° C. The temperature of the lake water employed was about 6° in midwinter.

It was necessary to supplement the heaters of the humidifier at the higher temperature. This was done by coiling upon the water feed-pipe a section of resistance wire which gave approximately 500 watts continuous service outside the insulation. Through the further action of the heaters in the conduits connecting humidifiers with culture chambers, the conditioned air was delivered into the latter at about 17° C. and 70 per cent relative humidity in one case, and 22° and 78 per cent relative humidity in the other. Thus there was approximated an atmospheric

temperature difference between the two chambers of 5°, while practically equal saturation deficits were maintained in the two cases. A comparison of saturation deficit with relative humidity is given by Livingston (*25*). The necessary computations are as follows:

Temperature.	Vapor pressure at saturation.[1]	Vapor pressure at relative humidity of—		Saturation deficit (by difference).
		70 per cent.	78 per cent.	
C.	*Mm.*	*Mm.*	*Mm.*	*Mm.*
17°	14. 53	10. 17	. . . . .	4. 36
22°	19. 83	. . . . .	15. 47	4. 36

[1] The values in this column are from Fowle (*6*).

As shown in Plate 1, B, where the black spores are conspicuous, the toweling was badly affected by molds. Addition of copper sulphate to the water stream proved ineffective after the organisms were established. From the experience of Morse with the destruction of copper-ferrocyanid membranes by molds (*28, p. 533*) there would seem to be little hope of avoiding difficulty by impregnation of the toweling with insoluble compounds of toxic elements. We are therefore substituting spray nozzles for humidification in future operation.

ILLUMINATION

Guided by exposure tests with photographic paper, additional shading was provided for the more westerly chamber, so as to equalize the solar radiation received by the two series of cultures, as indicated by the water losses from the black atmometers. The diminution of light intensity incident to the necessary shading of the chambers was partially compensated by placing a 500-watt, Mazda C, electric lamp over each. These were sufficiently distant to avoid serious heating effects and the light was concentrated upon the plants by conical reflectors. They were operated daily from about 5.30 p. m. to 9.30 p. m. and throughout cloudy days. Typical measurements of light intensity at the approximate level of the culture jars were obtained with a photometer. These appear in Table III.

TABLE III.—*Photometric values of light intensity in foot candles*

Character of day.	Location of test.			
	Shade of main house.	Isolated compartment south of chambers.	Within climatic chamber, lamp off.	Within climatic chamber, lamp on.
Clear	160	90	85	145
Cloudy	40	20	15	50

The beneficial effect of the artificial illumination was apparent in the growth response of the plants. Distinct etiolation of the latter became evident during early growth and before the lamps were installed.

With reference to the efficiency of the climatic apparatus as a whole, in view of the lack of refrigeration and other limitations it would be

manifestly unjustifiable to expect rigid control of atmospheric conditions by this installation. It was anticipated, however, that difference of evaporation could be restricted while maintaining a fairly constant temperature difference between the two culture chambers. It was evident that the plane of temperature in both chambers must be allowed to rise gradually as the season advanced into spring and the temperature of the lake water increased.

CULTURE CHAMBERS

The culture chambers were constructed from cypress 3 cm. thick in the form of four-paned window sashes as to sides and top. The sashes were set with glass of single thickness on both sides, thus providing heat insulation by an air space 1 cm. deep. The effective size of panes was 36.3 cm. square. Extra width of the bottom bars of the side sashes provided for isolation of a subchamber 10 cm. deep. Both floor and ceiling of this compartment were of 2 cm. pine boarding mortised to the sash bases. The culture chamber proper thus took the form of a cube with inside depth of 85.5 cm. It was painted glossy white throughout to promote reflection of light and heat.

A circular rotating table was provided in each chamber so as to facilitate uniform exposure of all cultures to varying degrees of temperature, humidity, and illumination. This was borne by a circular base of cast iron, 31.3 cm. in diameter, and projected upward in the form of a truncated cone. The base rested upon the bottom of the subchamber and its conical projection bore upon a ball bearing a cylindrical steel post 2 cm. in diameter and 10 cm. high. The latter was thus protruded through a central hole at the bottom of the culture chamber sufficiently to bear the rotating table. To it was fixed in the subchamber a wooden sheave 21.5 cm. in diameter and 2.5 cm. thick, by means of a central iron ring with set screw. The sheave was grooved for a small belt which passed through holes in the chamber wall to a reducing gear and motor outside. The table was composed of three pieces of cypress 2 cm. thick glued together to form a circular piece 75 cm. in diameter. It rested upon the supporting post through a centrally placed iron socket. Despite thorough painting it warped badly after some time. To avoid warping under these trying conditions, metal has been substituted for the wood.

One side of the chamber was supported upon hinges to allow access to both table and sheave. The conditioned air was conveyed from the heating cylinder outside to an opening beneath the edge of the rotating table by an extension of the sheet-iron cylinder projected diagonally upward on one side of the subchamber and converted to oval form as it opened into the culture chamber. To facilitate even distribution of the air upward, it was reflected beneath the rotating table by an arc of galvanized-iron sheeting erected close to the rim of the table from the base of the chamber. In addition, iron flanges were suspended radially at intervals from the bottom of the table. Air escaped from the chamber by a series of holes through the upper bars of the window sashes, whose total area was about 70 per cent greater than that of the intake.

SOIL CULTURES

On February 25, 1920, buckwheat seeds selected for uniformity of size from a Japanese variety were planted in 3.5 kgm. portions of Miami silt loam in earthenware jars (one-half gallon crocks). The soil was watered to 25 per cent of saturation, covered with paraffined paper, and placed

on the rotating tables. Each table bore six culture jars, a thermograph, a hygrograph, one white atmometer and one black one. The period of rotation was 40 seconds.

The seedlings appeared and were uncovered in 3 days at the higher temperature and one-half day later at the lower one. Thereafter the soils were brought to equal moisture content by weighing daily. On March 3 the number of plants per jar was reduced to 3 at the higher temperature, and a similar reduction was made at the lower temperature on March 5. On the latter date the plane of soil moisture was increased to 40 per cent of saturation. It was further increased to 50 per cent on March 19, and later reduced by two equal steps, on April 14 and 26, to 30 per cent. In the course of development aerial roots and red pigmentation appeared freely on the base of the stems for some distance

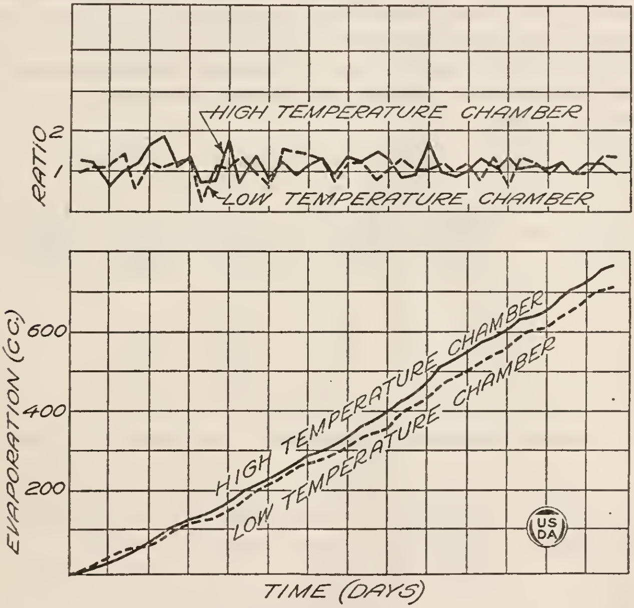

FIG. 1.—Relative evaporation (black atmometers) and relative solar radiation (ratio of evaporation between black and white atmometers). Climatic chambers, 1920.

above the soil. These features were especially prominent at the higher temperature.

On March 27, buds were unfolding at the higher temperature, although they were just appearing at the lower one. Several seeds had turned brown at the higher temperature on April 27, while those of the other cultures were still green. Photographs were taken on April 30. On May 10, the plants were harvested and separated into parts, as follows: leaf blades, petioles and stems, and seeds. These were dried at 100° C. and ground fine for chemical analysis. The data of climatic measurements and plant composition appear in Tables IV and V, while Plate 2, A, shows the appearance of the plants. Plate 3 shows the thermograph and hygrograph records. These are given for one week only, as it is hardly feasible to reproduce them in full. The graphs of figure 1 are constructed from the atmometric data.

Inspection of the climatic data shows that while the absolute extremes of temperature varied much more at the higher range than at the lower one, the daily average temperature varied only 4.5° in the former case and 3° in the latter. On the average, a difference of about 5.3° C. was maintained fairly continuously between the two chambers. The total evaporation during the growth of the cultures, as measured by the black atmometers, was only 6.5 per cent greater at the higher temperature than at the lower one. As measured by the white atmometers the difference of evaporation was 7.2 per cent. Had the mass of water remained the same at the former average temperature of 22.8° C. as at the latter average of 17.5° C, with the relative humidity at 70 per cent in the latter case, the saturation deficits would have been 10.32 mm. and 4.50 mm., respectively. Thus, if uncontrolled, the evaporation would have been 129 per cent greater at the higher than at the lower temperature. The normal tendency for difference in evaporation with uniform water supply was therefore greatly reduced in this experiment. As indicated by the ratio of water loss between the black and the white atmometers, the intensity of solar radiation was nearly the same in the two culture chambers.

TABLE IV.—*Climatic records of plant chambers in experiment of 1920*

Designation of temperature range.	Absolute maximum temperature.	Daily average maximum temperature.	Absolute minimum temperature.	Daily average minimum temperature.	Total evaporation standard black atmometer.	Ratio of evaporation black to white atmometer.
	°C.	°C.	°C.	°C.	Cc.	
High	33.0	25.0	10.0	20.5	769	1.083
Low	23.0	19.0	14.0	16.0	721	1.086

TABLE V.—*Yield and composition of buckwheat, soil cultures of climatic chambers in 1920*

Range of temperature (daily average).	Number of seeds at harvest.[1]	Dry matter of tissues.		Dry matter yield.[2]		
		Leaf.	Stem.	Leaf.	Stem.	Seed.
°C.		*Per cent.*	*Per cent.*	*Gm.*	*Gm.*	*Gm.*
20.5 to 25	73	14.7	11.2	5.36	9.52	2.03
16 to 19	57	15.9	13.9	5.26	9.15	1.82

Composition of dry matter.

Range of temperature (daily average).	Ether extract.			Polysaccharids.[3]			Nitrogen.		
	Leaf.	Stem.	Seed.	Leaf.	Stem.	Seed.	Leaf.	Stem.	Seed.
°C.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
20.5 to 25	9.7	1.0	3.2	18.2	33.0	39, 6	5.1	1.8	2.6
16.0 to 19	8.5	.9	3.2	22.6	38.2	45.4	4.5	1.4	2.8

[1] Broken parts removed before harvest contained 2 immature seeds at higher and 11 at lower temperatures.

[2] Dry weight of broken parts Leaf at high temperature 0.41 gm., low temperature 0.46 gm.; stem at high temperature 0.32 gm., low temperature 0.30 gm.

[3] Hemicellulose of leaf and stem determined by hydrolysis with 4 per cent HCl 3 hours; starch of seed determined by digestion with saliva.

As regards the yield of dry matter, when allowance is made for the loss of one plant at the lower temperature it is apparent that this factor was virtually constant at the different temperatures. That such correction should not be proportionate to the loss of plants in proportion to soil space is indicated by the work of Stewart (*37*). It should be noted that the discrepancy in numbers of seeds will be greatly reduced when one adds to those matured others involved in immature broken portions of the cultures. As the data stand, the weight per seed was somewhat greater at the lower temperature. In view of the conditions just mentioned, this difference can hardly be considered important, because of the lessened competition for nutrients as compared with the cultures bearing more mature seeds.

The plants grown at the lower temperature had a higher percentage of dry matter than the others, especially in the stems. The ether extract was more abundant percentagely in the leaves of plants grown at the higher temperature. This agrees with the deeper green color developed in this case, and is indicative of a relatively high chlorophyll content. The difference extended to the stems to a lesser degree, but disappeared in the seed, where the extract would be limited largely to true fats. A determination of the iodin numbers of the two fatty extracts of the seeds gave 55 per cent for the higher temperature cultures and 39.6 per cent for the others. However, the amount of material for analysis was too small to permit placing of emphasis upon these results.

Polysaccharids were more abundant throughout the plant in the cultures grown at the lower temperature, but the difference was somewhat less with the leaves than other tissues. In this connection, the work of Spoehr (*36*) should be noted. He found that with the approach of the dry season the cactus increased in pentosan content. The change was ascribed to a regulative mechanism for retention of water through production of polysaccharids of high capacity for hydration. Spoehr also concluded that a relatively high temperature (28° C.) produced the same effect, but his data are not so convincing as in the case of humidity effects. In an investigation of frozen peppermint Rabak (*30*) found evidence of increased esterification of menthol. A similar result was obtained by drying the plant tissue. Here, an extreme removal of water from liquid condition in the plant cells seems to have caused reversion of the familiar enzymic process of hydrolysis. It may well be questioned whether a similar effect would be likely to occur at temperatures much above freezing, but by hardening treatment with temperatures approaching freezing, Rosa (*33*) induced an increase of pentosan in certain plants.

It appears possible that the increase of polysaccharids observed in plants exposed to low temperature may bear some relation to disturbed equilibrium in the hydrolysis of these compounds. There are other possibilities, however, which should not be overlooked. One of these is the possible difference in net temperature coefficients for the synthesis of polysaccharids and of proteins in the plant. Another, and one rather more plausible than the others, is the possibility of limitation of polysaccharid storage due to consumption of sugars by increased respiration at higher temperatures. In this case the tissues would be expected to become richer in nitrogen, as is found by analysis.

With the exception of the seed, the nitrogen content of the several tissues of these buckwheat plants varied inversely as the polysaccharid content, but, even when expressed as equivalents of protein, they do

not compensate the variations of carbohydrates. In these cultures the modifying effect of varying temperature has been relatively free from disturbance by variations of either solar radiation or atmospheric humidity. Under these conditions an increase in polysaccharids has attended a decline of temperature value.

WATER CULTURES

Water cultures of buckwheat were conducted for a time parallel to the progress of the cultures just described. Seeds from the source previously used were suspended upon Shive's best solution for early growth of this plant, diluted to one-tenth the usual concentration. A group of seedlings were started in this manner in each chamber on March 15. Three seedlings were set up in each of four culture jars at the higher temperature on March 22, still employing one-tenth the usual concentration of Shive's solution. Two days later the seedlings were large enough at the lower temperature to be similarly transferred. At this time all of the nutrient solutions were made up to the usual concentration. On April 7, the third leaf was appearing in plants at the higher temperature, while the second leaf was just appearing in the other case. The leaves were greener and bases of the stems redder in the former case. Buds appeared on April 15 at the lower temperature and two days later at the higher one. On April 21, the solutions were changed to Shive's best proportions of salts for the last period of growth (35). The plants were harvested on April 26. At this time curling of the leaves and other indications of abnormal growth were becoming conspicuous, especially at the lower temperature. Only seven plants appeared reasonably normal in the latter case, and hence the seven best plants were selected from each series. The data of yield and composition appear in Table VI.

With the exception of the length of the tops, the physical measurements show little difference in the effect of the two temperature ranges upon the development of the plants. The slight difference in polysaccharid content varies in the same direction as with the soil cultures—that is, it was greatest at the lower temperature.

BUCKWHEAT IN SAND CULTURES WITHIN CLIMATIC CHAMBERS

Buckwheat was grown in sand cultures in the climatic chambers in 1921. This was for the purpose of avoiding possible modifying effects of temperature upon the fertility of soil, through action upon the soil organisms and in other ways. With the exception of using sand in place of soil and planting about two weeks later in the year, the experiment was conducted in essentially the same manner as the preceding one.

TABLE VI.—*Growth measurements and composition of buckwheat water cultures of climatic chambers in 1920*

Designation of temperature range.	Maximum length of tops.	Maximum length of roots.	Weight of dry matter.[1]	Composition of dry matter.	
				Ether extract.	Polysaccharids.[2]
	Cm.	*Cm.*	*Gm.*	*Per cent.*	*Per cent.*
High	48.8	19.6	0.72	4.4	24.7
Low	41.8	18.5	.82	4.6	25.4

[1] Total weights, including discarded plants, 1.13 gm. at high temperature and 1.19 gm. at low temperature.
[2] By boiling with 4 per cent HCl 3 hours after extraction of sugars and dextrins.

The sand was a mixture of 1 kgm. 100 mesh and 2 kgm. 50 mesh angular grains of quartz for each culture jar. It was rendered free of nutrients by extraction with hot 20 per cent HNO_3 and thorough washing, followed by leaching with lime water. The mixture had a water holding capacity of 42 per cent.

After standing in the climatic chambers a sufficient period to insure temperature equilibrium, the 12 jars of sand were planted on March 12, and watered to the extent of 10 per cent of the sand by weight. Radicles appeared above the sand on March 14, at the higher temperature and one day later in the other case. On the latter date the covers were removed and there were added 0.2 gm. $MgSO_4 \cdot 7H_2O$ and 0.4 gm. KNO_3 per jar, in solution.

The water plane of the sand was raised to 13.5 per cent on March 17. By March 21, higher percentage and vigor of germination were apparent at the lower temperature. On April 2, 16 of the 18 plants in this case were expanding the first true leaves, while only 8 plants had reached this stage at the higher temperature. A further addition of nutrients was applied per jar on April 4 as follows: 0.1 gm. $MgSO_4 \cdot 7H_2O$, 0.2 gm. KNO_3, and 0.01 gm. ferric citrate. The newer leaves were pale green at this time, and noticeably mottled at the higher temperature. On April 8, the moisture plane of the sand was raised to 17 per cent.

Buds appeared in both series of cultures on April 14, but they were more numerous at the higher temperature. On this date each jar received the following nutrients: 0.3 gm. $MgSO_4 \cdot 7H_2O$ and 0.6 gm. KNO_3. The final application of salts per jar was made on April 25, as follows: 0.6 gm. $MgSO_4 \cdot 7H_2O$, 1.2 gm. KNO_3, 0.39 gm. $Ca(NO_3)_2 \cdot 4H_2O$, 0.075 gm. KH_2PO_4, 0.075 gm. NaCl, and 0.015 gm. ferric citrate. At this time the plants at the lower temperature were uniform in size, while the other series was irregular in this respect. On April 29, the water supply was raised to 20 per cent of the sand, or about optimal for the plants.

Conspicuous differences in the reproductive phase of growth soon appeared. Thus on May 2 all of the plants at the lower temperature were in full bloom, while only a few plants were in bloom at the higher temperature. Thickening of the stems was rather prominent in the latter case. On May 6 several seeds were developed to considerable size at the lower temperature, while only one seed had appeared at the higher temperature by May 10.

After taking photographs on May 16, the cultures were harvested. Plants with only two true leaves or decidedly pale in leaf color were rejected. There remained 16 plants at the lower temperature and 13 at the higher. These were separated into leaves and stems, excluding the seed parts. The data of climatic factors and chemical analysis are assembled in Tables VII and VIII. The appearance of the plants is shown in Plate 2, B, while Plate 4 shows a portion of the climatic records. Graphs constructed from the atmometric data appear in figure 2.

Comparison of the climatic data of this experiment with that of 1920 was less satisfactory in the present case. This is to be ascribed largely to less effective functioning of the humidifiers, due to deterioration of the toweling. In future development of the apparatus humidification will be accomplished by means of spray nozzles. The variation of the lower temperature range here was greater than that of the upper range, while the reverse was true in the earlier experiment. As a general average, the temperature was maintained at about 5°C. between the temperature planes here, but the average planes of operation were about

2.5° higher than in the earlier experiments. The attempt to check evaporation at the higher temperature was overdone, so that evaporation, as measured by the black atmometer, was 10 per cent less than at the lower temperature. The ratio of water losses from the black and

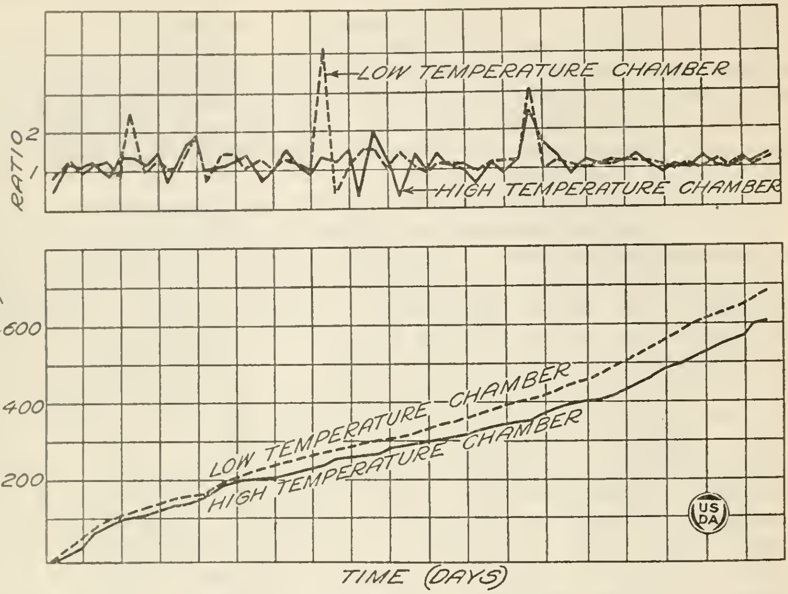

FIG. 2.—Relative evaporation (black atmometers) and relative solar radiation (ratio of evaporation between black and white atmometers). Climatic chambers, 1921.

white atmometers indicate practically equal illumination in the two culture chambers.

TABLE VII.—*Climatic records of plant chambers in experiment of 1921*

Designation of temperature range.	Absolute maximum temperature.	Daily average maximum temperature.	Absolute minimum temperature.	Daily average minimum temperature.	Total evaporation standard black atmometer.	Ratio of evaporation black: white atmometer.
	°C.	°C.	°C.	°C.	Cc.	
High	34.0	28.2	18.5	23.2	610	1.085
Low	30.0	23.3	14.0	16.9	678	1.085

TABLE VIII.—*Yield and composition of buckwheat, sand cultures of climatic chambers in 1921*

Range of temperature (daily average).	Number of seeds at harvest.	Dry matter yield.[1]		Ether extract.		Composition of dry matter. Polysaccharids.[2]		Composition of dry matter. Insoluble nitrogen.[3]	
		Leaf.	Stem.	Leaf.	Stem.	Leaf.	Stem.	Leaf.	Stem.
°C.		Gm.	Gm.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
23.2 to 28.2	0	0.47	0.66	6.6	4.5	18.3	14.8	2.3	0.7
16.9 to 23.3	6	.50	.79	7.1	4.1	17.9	19.6	2.5	.8

[1] Thirteen plants at high temperature, 16 at low temperature.
[2] Determined by boiling with 4 per cent H_2SO_4 for 2.5 hours after extraction of sugars and dextrins.
[3] Determined on the residue from acid hydrolysis with one-half the acid extract added.

When the relative numbers of plants selected are considered, it appears that the development of dry matter in the leaves was appreciably lower at the lower temperature. The generally greater development of height of plants at the higher temperature is shown in Plate 2, B. Only well-filled and apparently normal seeds are considered in the data. On this basis there was a marked deficiency of the reproductive function at the higher temperature. This merits attention in connection with the chemical composition of the plants. With regard to the latter factor, the only distinct difference is in the polysaccharid content of the stems. In this respect the experiment agrees with the one conducted upon soil in 1920, in that the plants grown at the lower temperature contained about 5 per cent more of this constituent. It seems desirable to suggest that, in connection with the limited general development of these cultures, those at the higher temperature may have been unfruitful because of an unfavorable balance between nitrogen and carbohydrate content, according to the conclusions of Kraus and Kraybill (*16*).

SUMMARY

(1) A brief digest of the literature has shown variations of form and composition of plants in response to variations of climatic factors. In certain cases the decrease of temperature appears to have been specifically associated with increase of polysaccharids in the plants. The importance of these relations to problems in physiology is mentioned.

(2) Red clover (*Trifolium praetense*) grown in two greenhouses at 15° and 20.6° C. average temperatures, with constant soil water supply, but with 47 per cent excess of evaporation at the higher temperature and 44 per cent excess of solar radiation at the lower one, contained about 4 per cent more of polysaccharids in the tops of the plants grown at the lower temperature than in the other case. The crude protein content of the plants was least at the lower temperature, but not in proportion to the difference of polysaccharids.

(3) Chambers for the control of atmospheric temperature and humidity are described.

(4) Buckwheat (*Polygonum fagopyrum*) grown in soil cultures with a uniform supply of soil moisture at average atmospheric temperatures of 17.5° and 22.8° C., with evaporation 7.2 per cent greater at the higher temperature than at the lower one and with the reinforced solar radiation 3.6 per cent greater in the latter case, contained 5.8 per cent more starch in the seeds and 5.2 per cent more polysaccharids in the stems at the lower, as compared with the higher, temperature. The nitrogen contents of the stems and leaves varied inversely as the polysaccharid contents, but not proportionately so.

(5) Buckwheat (*Polygonum fagopyrum*) grown in sand cultures with uniform supplies of water and nutrient salts at average atmospheric temperatures of 20.1° and 25.7° C., with evaporation 10 per cent less at the higher temperature than at the lower one, and with reinforced solar radiation equal in the two cases, contained 4.8 per cent more polysaccharids in the stems at the lower temperature than at the higher one. The plants grown at the higher temperature produced no seeds of normal appearance.

(6) From the results herein presented, it appears that independent of its indirect effects through modifying the soil and independent of certain variations of atmospheric humidity and total magnitude of exposure to

solar radiation, atmospheric temperature modifies the percentage of polysaccharids in tissues of the plants here tested.

(7) Suggestions are offered as to the possible mechanisms by which the temperature effect here observed may be consummated.

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PLATE 1

A.—Climatic chambers in operation.

a. Water supply to humidifier.
b. Heating units of humidifier.
c. Mercury thermostat of humidifier.
d. Bimetallic thermostat of culture chamber.
e. Atmometer on rotating table.
f. Hygrograph and thermograph on rotating table.
g. Air conduit with gate.
h. Motor and reducing gears belted to rotating tables.
i. Electric lamps.

B.—Humidifier.

a. Asbestos blanket.
b. Toweling, showing brass clips in tray above.
c. Concentric cylinder to contain heating units for water supply.
d. Fan supplying air.
e. Front flanges directing air current.
f. Escape to drain.

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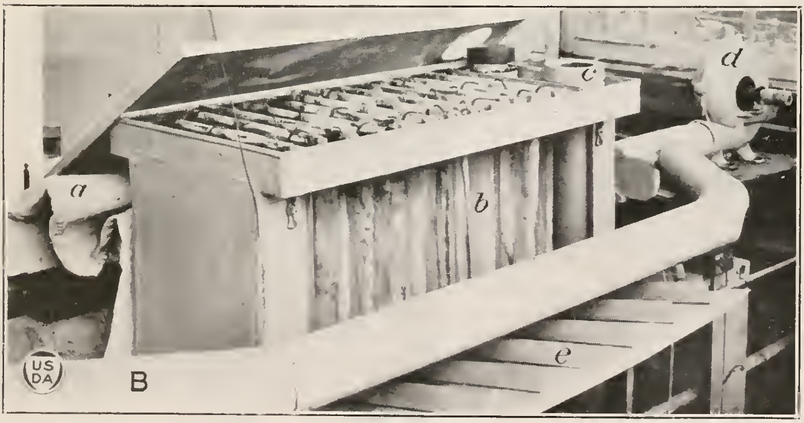

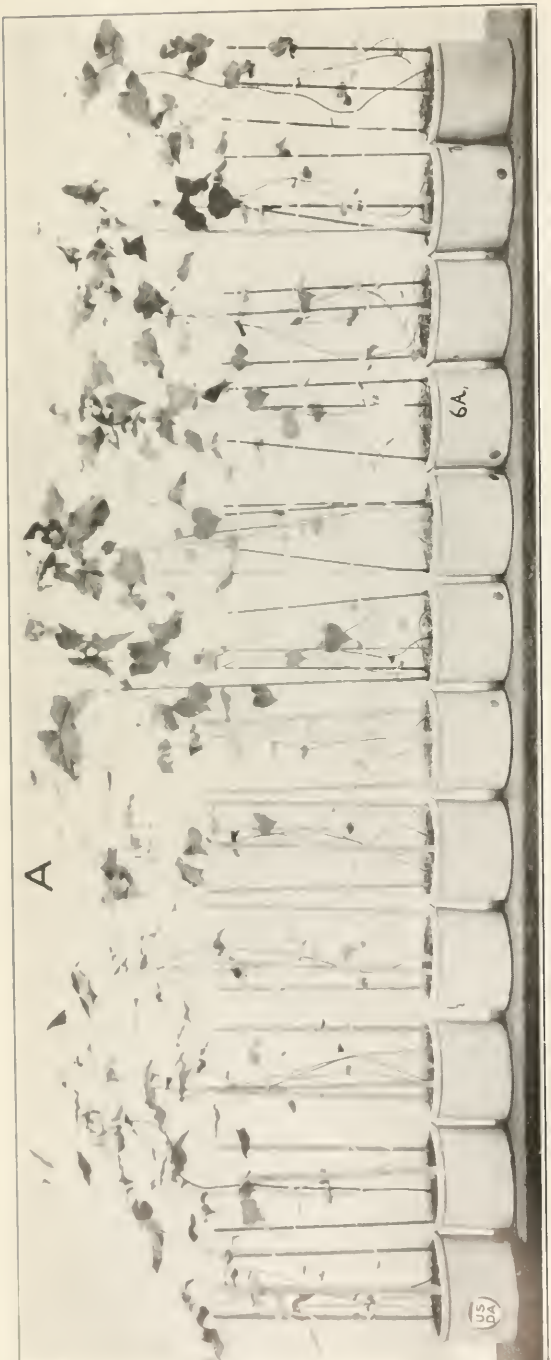
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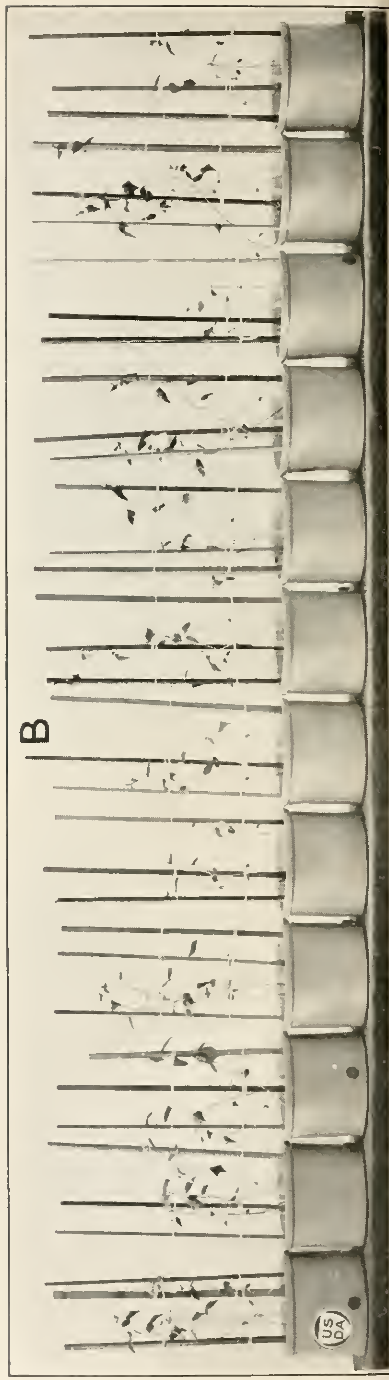
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PLATE 2

A.—Cultures of buckwheat on soil in climatic chambers in 1920.
Six left-hand cultures at lower temperature.
Six right-hand cultures at higher temperature.

B.—Cultures of buckwheat on sand in climatic chambers in 1921.
Six left-hand cultures at lower temperature.
Six right-hand cultures at higher temperature.

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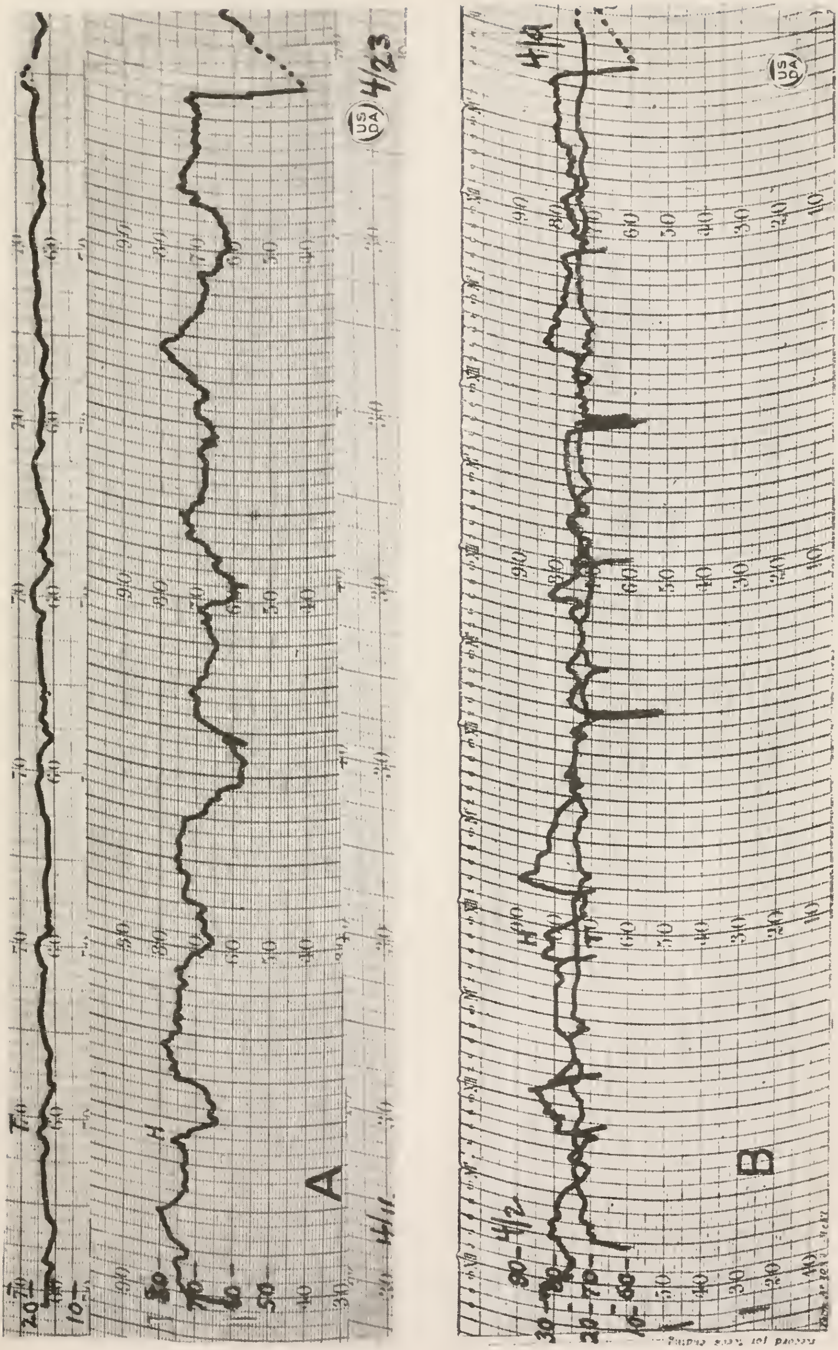
PLATE 3

A.—Climatic records, low temperature chamber, week beginning April 2, 1920.
B.—Climatic records, high temperature chamber, week beginning April 2, 1920.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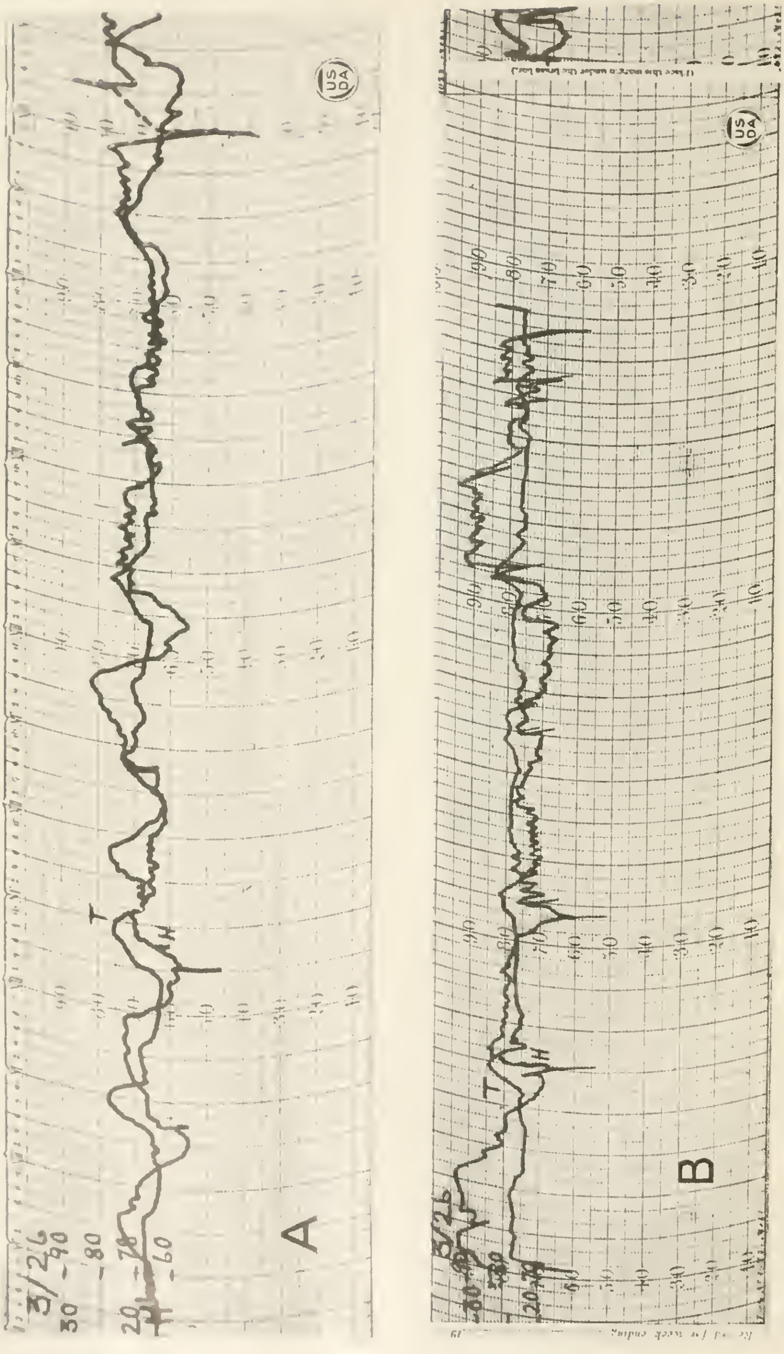
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B

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PLATE 4

A.—Climatic records, low temperature chamber, week beginning March 26, 1921.
B.—Climatic records, high temperature chamber, week beginning March 26, 1921.

31

PLATYGASTER VERNALIS MYERS, AN IMPORTANT PARASITE OF THE HESSIAN FLY[1]

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INTRODUCTION

In the fall of 1914, W. R. McConnell and P. R. Myers undertook an exhaustive study of the Hessian fly (*Phytophaga destructor* Say) at Hagerstown, Md., with particular reference to its parasites. Since that time more or less consecutive records have been kept of the occurrence of one of these parasites, *Platygaster vernalis* Myers,[3] throughout the eastern wheat-growing region. In 1917 the laboratory at Hagerstown was transferred to Carlisle, Pa., and in this year the writer joined in the investigations. During the course of these studies *P. vernalis* was first recognized and described by Mr. Myers,[4] but this parasite was not studied intensively until 1918, when the writer undertook to discover the details of its life history. This work, with many interruptions, has continued to date. The present paper summarizes the data collected on this parasite since 1914.

ECONOMIC IMPORTANCE

From the standpoint of economic importance *Platygaster vernalis* stands first among the many species of parasites that normally attack the spring generation of the Hessian fly in the Middle Atlantic States. Percentages worked out for various species of Hessian fly parasites have shown that *P. vernalis* is more effective than any other species attacking the spring generation of the fly in this region.

Table I.—*Percentage of Hessian flies killed by Platygaster vernalis for the years 1915 to 1920, inclusive, together with the total puparia examined in order to obtain these data, with the average and total for the entire period*

Year.	Percentage killed by vernalis.	Number of puparia examined.
1915	40.10	2,582
1916	15.53	2,285
1917	15.73	2,143
1918	19.99	6,930
1919	24.68	2,297
1920	27.34	2,419
Average	23.89	18,656

[1] Accepted for publication Aug. 15, 1922.

[2] The writer wishes to express his appreciation of the assistance rendered by the late W. R. McConnell and by P. R. Myers in contributing useful suggestions during the progress of the work and in helping to rear and determine much of the material used; he also wishes to thank Dr. R. W. Leiby for helpful criticisms, Messrs. R. M. Fouts and H. D. Smith for the determination of many of the parasites, and Miss Esther Hart for the drawings of the adult, head, and antennæ.

[3] Order Hymenoptera, superfamily Serphoidea, family Platygasteridae.

[4] Myers, P. R. A new American parasite of the Hessian fly (Mayetiola destructor Say). *In* Proc. U. S. Nat. Mus., v. 53, p. 255-257. 1917.

An average of 23.89 per cent of the spring generation of the host is destroyed annually by *Platygaster vernalis* throughout this territory. This statement is based on the examination of 18,656 puparia collected during a period of six years (1915 to 1920, inclusive) from 39 well separated localities ranging from Montoursville, Pa., on the north, to Staunton, Va., on the south. Table I gives the percentage for each of these years and the average for the period.

DISTRIBUTION

Platygaster vernalis has been found throughout the eastern wheat-growing region as far north as 43° 33′ and as far south as 37° 50′ north latitude. Considerable material collected at Evans Mills and Theresa, N. Y. (a little north of the forty-fourth parallel), revealed no *vernalis* present. No abundance of *vernalis* was found farther south than Staunton, Va., a short distance north of the thirty-eighth parallel. This species was not found in material collected at Lexington, Va., about 35 miles southwest of Staunton, but one specimen was reared from material taken at Buchanan, about 20 miles southwest of Lexington, latitude 37° 50′. In the Middle West *P. vernalis* has been found in abundance at Wanatah, Ind., and records have been made of its occurrence at Niles, Mich., Strongville, Wooster, Troy, and Columbus, Ohio, and Charleston, Mo.

THE EGG

The egg is highly refractive, claviform in shape, and before oviposition measures about 0.07 millimeter long by 0.016 millimeter wide. (Pl. 1, A, B.) A minute projecting piece of membrane may sometimes be found at the swollen extremity. Immediately after oviposition the main body of the egg (Pl. 1, B) is usually found dilated to about twice the width mentioned. Plate 1, A, shows a camera-lucida sketch of an egg freshly removed from the ovary, and Plate 1, B, represents an egg immediately after it was oviposited into the host egg.

The egg never develops except in the midintestine of the host. Surrounded by the chyle of the stomach, it is tossed about by peristaltic action. Plate 1, C, shows a Hessian fly larva in longitudinal section with a single *vernalis* egg about 22 days old submerged in the chyle in the midintestine.

The germ cell of the egg, instead of developing as a single embryo, as is the case with most insects, gives rise to several embryos. The nutritive plasm also develops precociously. Plate 1, D, shows a parasite body about 11 days old in sagittal section with four embryos in the blastula stage of development. Paranuclear masses may be seen scattered irregularly about in the surrounding plasm. Plate 1, E, shows a parasite body containing eight embryos that are much further advanced. Each embryo is surrounded by an individual membrane and the surrounding plasm is gelatinous in consistency. Paranuclear masses are still present.[5]

The illustration shows the entire mass somewhat flattened out. In the host's stomach it tends to assume a spherical shape, but its plastic nature permits it to be compressed into various shapes by the peristaltic action.

The number of embryos found to develop from a single egg ranges from 2 to 12.

[5] The outlines of the sketch were made by camera lucida immediately after the embryonic mass had been taken from the host body and brought into normal salt solution. Later the embryonic mass was stained in picrocarmine to bring out the cell structure, and greater details of the sketch were taken from these stained and mounted embryos.

THE PRIMARY LARVA

In general outline the primary larva (Pl. 2, A) is elongate oval, with length of body about three times its width and with a very slight taper toward the caudal end. Both extremities are bluntly rounded, and before the larva has become very much inflated with food a broad, deep constriction is evident on each side slightly posterior to the mouth. This constricted appearance is due to the greatly enlarged and projecting bases of the mandibles.

The mandible (Pl. 2, B) is noticeably long, being a little over one-third the width of the head. Measurements of single mandibles from four larvæ show an average length of 0.073 millimeter. The mandible is wide at the base but tapers to a sharp extremity, with the distal third slightly curved. Several long, closely adhering spines are discernible along the curved portion and at the extremity. The entire mandible is nearly colorless and comparatively fragile. The mouth consists of a small transverse aperture, capable of being opened and closed by motion of the superior lip.

The larva is usually sufficiently transparent to disclose under proper magnification certain outstanding features of the internal anatomy, such as the cells of the stomach wall and epithelium. Some of the cellular structures of the internal anatomy, including the stomach wall and proctodaeum, are illustrated in Plate 2, A. The details of cell structure were taken from microtomic sections.

When the larva has developed sufficiently to feed, a movement of the labrum begins, thus producing a suction whereby the surrounding liquids or adjacent tender tissues are ingested. After freeing itself from the surrounding embryonic mass the larva first imbibes the green chyle from the host's stomach and soon ingests particles of the stomach wall itself. While it may possibly secrete juices which have a softening effect on surrounding tissues, nevertheless undissolved particles of host tissue within the stomach of the parasite have been clearly seen, and in one case part of the stomach tissues of the host was observed protruding from the parasite's mouth.

Frequently the number of young larvæ found in a single host much exceed the number that ever reach maturity. Single hosts have been found to contain 21, 27, 32, 34, and 40 young larvæ. In several instances 1 or 2 of such larvæ were found in a stunted condition while the others in the same host were normal. In one host a single dead larva was discovered, while the others appeared normal and healthy. In another case 5 partially developed young larvæ (2 of these exceptionally large ones), and 4 very small, poorly developed ones were found. At times all the young larvæ were found dead within the host. Where this occurred the cause frequently was found to be hyperparasitism by undetermined chalcidoids.

THE MATURE LARVA

The mature larva (the lateral aspect of which is shown in Pl. 2, C) is about 1 millimeter long by 0.5 millimeter thick. It is white, ovoid, bare of setæ, and with 11 clearly defined body segments. Spiracles are present on the second and third thoracic segments and second abdominal segment only. In the first abdominal segment, instead of an external spiracle, a large discoidal body occurs under the cuticle at the terminus of the lateral tracheal branch of this segment. The mouth (Pl. 2, D) is a small

transverse orifice, when closed appearing as a somewhat crescent-shaped slit and when open forming an oval-shaped aperture. In the process of feeding, the superior lip is moved toward and away from the inferior lip by strong radiating muscles. The inferior lip is slightly thickened along its rim. On each side slightly posterior to the mouth is a small, distinctly curved mandible. (Pl. 2, E.) The mandibles are widely separated from each other, distinctly chitinized, and less than half the length of the mandible of the primary larva. The average length of 10 mandibles from 10 mature larvæ was found to be 0.03 millimeter. Slightly caudad of the mandibles is a pair of faintly chitinized maxillæ (Pl. 2, F), which are rather broad at the base and taper slightly to a blunt point. The maxillæ are slightly longer than the mandibles, with the inner side a little shorter than the outer.

During this stage of the parasite, the remainder of the host is consumed as far as the cuticula, which is left to inclose the cocoons that subsequently are formed.

THE COCOON

The cocoon (Pl. 3, A) is broadly ellipsoidal and of a flexible, smooth, shiny, yellowish brown consistency. The cuticula of the host is left intact, but adheres so tightly to the cocoons as to appear as part of them and doubtless serves as a protection to them. In Plate 3, A, the external spatula of the host may be seen adhering at one extremity. From 3 to 13 such cocoons have been found in single hosts, and the examination of 100 hosts showed an average of 7.91 cocoons per host. The host invariably formed its brown puparium case before being killed by the larvæ of *Platygaster vernalis*, thus providing the hibernating parasites an additional protection.

THE PUPA

The pupa (Pl. 3, B), when first formed within the cocoon, is white, but the compound eyes soon darken and gradually the entire body turns a shiny black with the exception of the thin integument between the abdominal plates. The pupal stage is shorter in duration than either the larval or the adult stage.

THE ADULT

DISTINGUISHING MORPHOLOGICAL CHARACTERISTICS

The adult (Pl. 4, A) is from 0.7 millimeter to 0.9 millimeter long' with shining black body. Certain distinguishing characteristics are as follows:

Head quadrate, about as wide as thorax; face convex; occiput, vertex, and face distinctly transversely rugulose (Pl. 4, B); antenna with base of scape black, second joint of the flagellum in male distinctly curved and larger than that of the female (Pl. 4, C and D); scutellum laterally margined; legs entirely piceous; tarsi fuscous; wings 2½ to 3 times as long as abdomen; ovipositor (Pl. 3, C and D) straight, slightly enlarged and blunt at apex. Plate 3, D, shows the sheath, gorgeret, and stylet of the ovipositor. The ovaries are also characteristic of the species, being nearly spherical, with short, thick oviduct about as long as the diameter of the ovary. A single ovary is shown in Plate 3, E.

PARTHENOGENESIS

Experimental rearings have shown that parthenogenesis may occur in *Platygaster vernalis*. In one experiment seven unfertilized females were put under a cloth-topped glass cylinder on potted wheat plants bearing eggs of the Hessian fly on their leaves. The Hessian fly larvæ which

developed from these eggs were killed on different dates and sectioned. Three such larvæ killed at the end of 26 days contained normally developing *vernalis* embryos; two larvæ killed at the end of 43 days disclosed the presence of healthy, mature *vernalis* larvæ; and two killed at the end of 78 days contained *vernalis* cocoons. Two similar cages showed the development of healthy *vernalis* embryos.

SEX RATIO

From 1,169 adults which emerged in confinement, 48.59 per cent were females and 51.41 per cent males. The occurrence of parthenogenesis may explain the preponderance of males in this species.

To determine whether the several individuals developing in single puparia usually comprised one or both sexes, records were kept of the sex of the adults (whether emerged or not), in the case of 48 host puparia. Of these 48 puparia, 40 yielded adult parasites, each brood of which was either pure male or pure female; while each of the remaining 8 puparia produced a mixed brood of both males and females. This indicates that in the polyembryonic development of *Platygaster vernalis* the adults produced from a single egg are usually of the same sex. The coming of both sexes from a single host could be explained on the grounds that more than one egg was deposited in a single host insect.

OVIPOSITION

A female of *Platygaster vernalis*, when seeking Hessian fly eggs in which to oviposit, travels at a moderate rate up and down the leaves of the wheat plant, repeatedly tapping the leaf before her with her antennæ.

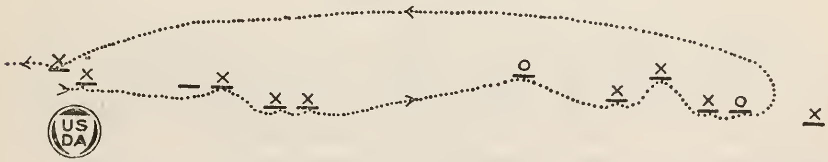

Fig. 1.—Diagram showing the tendency of *Platygaster vernalis* to avoid ovipositing twice into the same egg. The short lines represent the Hessian fly eggs; the crosses mark out the eggs oviposited into on the first visit of the parasite; the dotted line plots out the course of the second visit; the circles mark out the host eggs oviposited into during the second visit.

As soon as her antennæ come in contact with an egg, she halts and concentrates on the spot. At times she loses track of the egg, whereupon she turns in small circles until she finds it again. After finding the egg, the parasite strokes it rapidly with her antennæ and meanwhile strides it with her body held parallel to the long axis of the egg, whereupon the abdomen is drawn up with the ovipositor exserted and in contact with the egg. After sliding the ovipositor back and forth over the surface of the egg several times, she begins to insert it, usually at about the middle of the egg. At this point she draws her antennæ downward and remains motionless for the rest of the time, except for slight movements of her abdomen, while bearing down on her ovipositor. The entire act of oviposition requires about one minute, the average of 10 ovipositions being 59.9 seconds.

Although certain individuals in confinement have deliberately oviposited several times in one host egg, most females seem to avoid ovipositing more than once in the same egg. This tendency is shown by the experiment illustrated in figure 1.

In this diagram 12 Hessian fly eggs are represented in approximately the same position as they occurred on the wheat leaf. A *vernalis* female was allowed to visit these eggs twice. During the first visit the eggs marked with a cross were attacked. The dotted line plots out the course of the second visit, and the circles indicate the eggs attacked during this trip. During the second trip the female examined each of the eggs with the exception of the one at the extreme end, but she consistently refused to oviposit in any except two which had been omitted during the first trip.

Experiments indicate that *Platygaster vernalis* lays only one egg during a single oviposition. This is the usual number found in reared material and in that collected in the field. In confirmation of this belief, it was found that 11 host eggs laid in confinement and dissected immediately after having been punctured by this species contained but one *vernalis* egg each.

POTENTIAL PROGENITIVENESS

At the time of eclosion of the adults the eggs have reached their full development in size. In order to ascertain the average number of eggs contained in the ovaries, the eggs of 10 females were dissected out and counted. The count showed an average of 228.3 eggs per female, with a maximum of 290 and a minimum of 117. The eggs are so small and numerous that in order to count them it was necessary to spread them out in a liquid on an eye-piece micrometer disk ruled into 1 millimeter squares. By counting the eggs from one ovary at a time, accurate results were possible.

LENGTH OF LIFE

In order to obtain data on the length of life of the adult, 59 adults were divided into three groups subject to different conditions. Lot 1 included 15 females and 4 males, placed in a large dry vial plugged with cotton and with a little sugar solution for nourishment. Lot 2 contained 14 females and 8 males in small vials plugged with cotton and left in a saturated atmosphere, with water accessible. Lot 3 consisted of 15 females and 3 males in small vials in a saturated atmosphere but with sugar solution for nourishment. The atmosphere for Lots 2 and 3 was kept saturated by placing the vials in a relaxing box. The results are summarized in Table II. The shortest length of life was 3 days and the longest 27 days; in Lots 1 and 2 the average length of life of the females exceeded that of the males; and the parasites left in a saturated atmosphere with water available had the longest average length of life, namely, 12.21 days. Throughout the experiment the temperature of the laboratory in which it was conducted varied from 45° F. to 78° F., with an average temperature of 61° F.

Extremely low or high temperatures undoubtedly have some effect on the length of life. Several adults, however, were subjected to a temperature of 24° F. (−4.45° C.) for a period of 5 hours without any noticeable ill effects. Adults subjected to heat expired within a minute at temperatures from 117.68° F. to 120.2° F. (47.6° C. to 49° C.). Short exposures to temperatures of 117.22° F., 116.60° F., and lower did not prove fatal.

TABLE II.—*Length of life of Platygaster vernalis adults*

[Lot 1 was kept in a dry atmosphere with sugar solution for nourishment; Lot 2 in a saturated atmosphere, with water; and Lot 3, in a saturated atmosphere, with sugar solution for nourishment]

Lot.	Number of adults.		Length of life in days.				
	Female.	Male.	Maximum.	Minimum.	Average.		
					Female.	Male.	Total.
1	15	4	6	3	4.53	3	4.15
2	14	8	26.33	3	14.71	9.61	12.21
3	15	3	27	3	8.46	8.89	8.53

OTHER BEHAVIOR

When in confinement the adults crawled rapidly about but very seldom flew. They nearly always moved toward the light. When suddenly disturbed, they feigned death by drawing up the legs and antennæ close to the body and remaining in this attitude for a few seconds. In actual death, the antennæ and legs are found stretched away from the body. When at rest, the body is usually held in a crouched position, but with legs and antennæ not drawn as close to the body as when simulating death.

For nourishment the adults readily take up sugar solution. When allowed to go a few days without water, they became very thirsty, as was demonstrated by the quickness with which they found a drop of water placed near them and the eagerness with which they accepted it.

When emerging, the adult parasite gnaws a round or irregularly shaped exit hole through the cocoon and puparium. (Pl. 3, F.) Sometimes a few such holes in the host puparium are sufficient to permit all the adults within the host to escape.

SEASONAL HISTORY

To obtain data on the duration of the various stages of *Platygaster vernalis* under normal field conditions, dissections were made of the Hessian fly in various stages collected at intervals throughout the year. Such records were kept during 1918 at Carlisle, Pa., 1919 and 1920 at Mount Holly Springs, Pa.; and 1921 at New Windsor, Md.

The lines on figure 2 show the maximum range of occurrence of each stage as determined by the earliest and latest records of their presence in the field, assembled from data collected during the four years mentioned. The records of the occurrence of adults were obtained by sweeping, and those of the embryos from dissections of Hessian fly larvæ after they had descended to the bases of the plants. Since *Platygaster vernalis* oviposits in the egg of its host, embryos of this parasite must have occurred in the field somewhat earlier than is indicated in the figure. It may be observed that the *vernalis* larvæ spend considerable time within cocoons before pupating. Cage rearings, checked by field examinations made during the winter, show that the adults normally remain within the cocoons until early spring. The line representing this stage on the chart has not been extended farther than to September 27 for economy

of space. Occasionally adults have been found to emerge and oviposit during the autumn, but this is exceptional, and field observations indicate that eggs deposited at that season fail to mature.

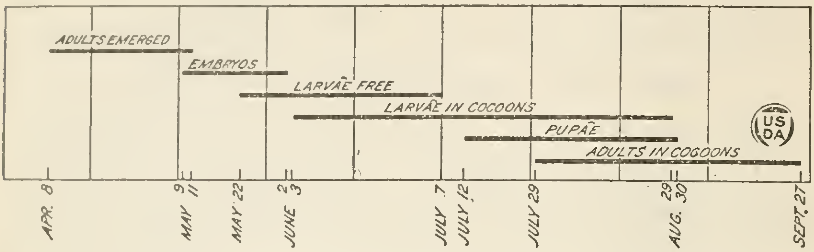

FIG. 2.—Periods of occurrence of the various stages of *Platygaster vernalis* as found from examinations made during the years from 1918 to 1921, inclusive. The dates on which the examinations were made are indicated at the bottom of the diagram.

Figure 3 shows the rate of development of the various stages of *Platygaster vernalis* from May 17 to October 18. These data were obtained by the dissection of Hessian fly stages collected in lots of 100 or more

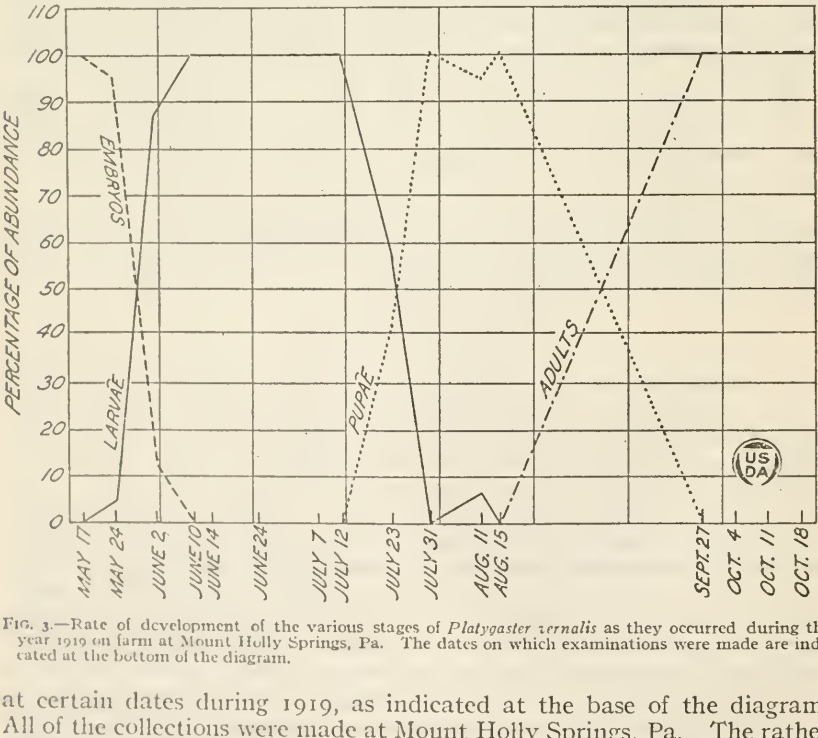

FIG. 3.—Rate of development of the various stages of *Platygaster vernalis* as they occurred during the year 1919 on farm at Mount Holly Springs, Pa. The dates on which examinations were made are indicated at the bottom of the diagram.

at certain dates during 1919, as indicated at the base of the diagram. All of the collections were made at Mount Holly Springs, Pa. The rather late occurrence of *vernalis* adults during that year was probably due entirely to meteorological conditions.

CERTAIN ECOLOGICAL CONSIDERATIONS

Various factors in the environment of *Platygaster vernalis* have a marked effect in modifying its normal rate of multiplication and help to produce a correspondingly high or low annual rate of mortality.

By making a series of examinations of Hessian fly forms taken from one certain field or farm throughout the season, it has been possible to obtain interesting data bearing on this point. The individual puparia in each examination were selected impartially as they were met with, while the wheat tillers were being inspected for them. They were dissected under the binocular microscope and the contents of each carefully classified. Table III shows the results obtained during the summers of 1918, 1919, 1920, and 1921. The same results are graphically represented in figures 4, 5, 6, and 7. The collections were made on a farm at Carlisle, Pa., in 1918, on a farm at Mount Holly Springs, Pa., in 1919 and 1920, and on a farm at New Windsor, Md., in 1921.

TABLE III.—*Mortality of Platygaster vernalis, as observed in collections of Hessian fly forms made at intervals during the years 1918, 1919, 1920, and 1921*

Date of collection.	Fly forms examined.	Results of examination of Hessian fly forms. Containing living *P. vernalis*.	Containing dead unrecognizable matter and dead *P. vernalis*.	Otherwise parasitized.	Living unparasitized fly forms.	Mortality of *P. vernalis*.
1918: FIVE COLLECTIONS FROM FARM, CARLISLE, PA.						
	Number.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
May 7	58	79.31	0.00	0.00	20.69	0.00
June 17	184	76.09	10.33	4.89	8.69	4.06
24	52	59.62	15.38	7.69	17.31	24.83
July 19	18	44.44	.00	50.00	5.56	43.96
Aug. 27	81	14.81	28.40	53.09	3.70	81.33
1919: NINE COLLECTIONS FROM FARM, MOUNT HOLLY SPRINGS, PA.						
May 27	139	57.55	1.44	0.00	41.01	0.00
June 5	100	46.00	4.00	4.00	46.00	20.07
12	110	28.18	15.46	8.18	48.18	51.03
July 11	100	23.00	21.00	36.00	20.00	60.03
21	100	22.00	23.00	46.00	9.00	61.77
Aug. 7	150	12.67	27.33	52.67	7.33	77.98
14	200	11.00	25.00	60.00	4.00	80.89
Sept. 25	200	4.00	29.50	62.50	4.00	93.05
Oct. 10	200	3.50	27.50	66.50	2.50	93.91
1920: FIVE COLLECTIONS FROM FARM, MOUNT HOLLY SPRINGS, PA.						
June 10	14	51.00	6.00	7.00	36.00	0.00
July 21	186	7.51	40.86	50.55	1.08	85.27
Aug. 31	200	9.00	51.00	38.00	2.00	82.35
Sept. 8	100	4.00	51.00	45.00	.00	92.16
Nov. 27	100	2.00	55.00	42.00	.00	96.08

TABLE III.—*Mortality of Platygaster vernalis, as observed in collections of Hessian fly forms made at intervals during the years 1918, 1919, 1920, and 1921*—Continued

1921: EIGHT COLLECTIONS FROM FARM, NEW WINDSOR, MD.—Continued.

Date of collection.	Fly forms examined.	Results of examination of Hessian fly forms.				
		Containing living *P. vernalis.*	Containing dead unrecognizable matter and dead *P. vernalis.*	Otherwise parasitized.	Living unparasitized fly forms.	Mortality of *P. vernalis.*
	Number.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
May 9	117	76.07	15.39	0.85	7.69	0.00
June 3	100	63.00	21.00	16.00	.00	17.18
21	100	29.00	23.00	40.00	8.00	61.88
July 12	100	16.00	43.00	38.00	3.00	78.97
28	100	16.00	56.00	26.00	2.00	78.97
Aug. 12	110	20.91	46.36	28.18	4.55	72.51
30	100	21.00	47.00	29.00	3.00	72.39
Sept. 23	100	6.00	70.00	21.00	3.00	92.11

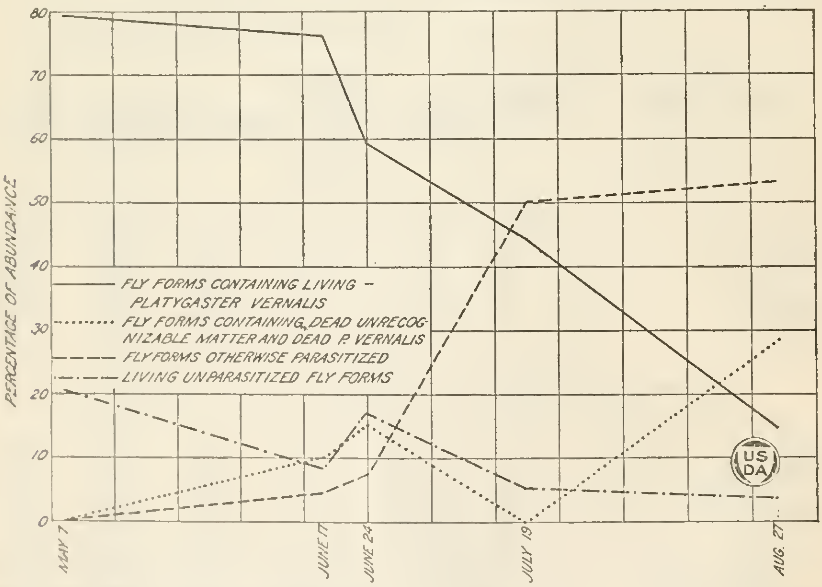

FIG. 4.—Mortality of *Platygaster vernalis*, as observed in collections of Hessian fly forms made at Carlisle, Pa., in 1918. (Table III.)

In all cases, for the sake of the uniformity necessary in obtaining correct percentage results, the host is taken as the unit. For instance, if all the individual parasites in a single host were dead, the latter was classified as containing dead *vernalis*, but if one or more of the *vernalis* were alive, the host was classified as containing the living parasite.

Under the heading "Otherwise parasitized" are included puparia from which parasites other than *vernalis* have emerged, or in which such other parasites are recognized whether dead or alive. In case of the recognizable occurrence of both *vernalis* and some other parasite in the

same host, it was classified according to the condition of *vernalis*. Such cases were comparatively few.

The number of hosts containing dead material recognized as *vernalis* was, as a rule, exceedingly small. The cause of the death of *vernalis* in such cases could seldom be determined, although sometimes they appeared to have been eaten by predators.

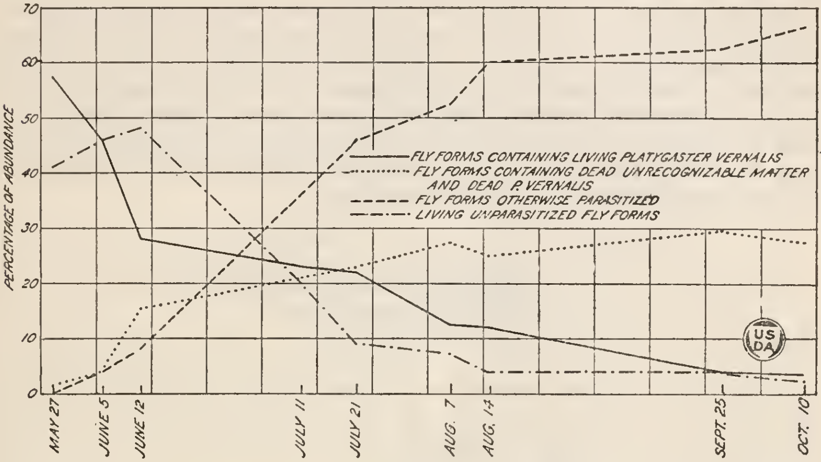

FIG. 5.—Mortality of *Platygaster vernalis* as observed in collections of Hessian fly forms made at Mount Holly Springs, Pa., in 1919. (Table III.)

In 1918 (Table III), by the 19th of July, the mortality of *Platygaster vernalis* reached 43.96 per cent and by August 27, the date of the last collection for that year, it had reached as high as 81.33 per cent. At that time the death of at least 45.51 per cent of the *vernalis* parasites was due to the competition of other Hessian fly parasites. It would be hazardous to say that any greater proportion were destroyed by this agency,

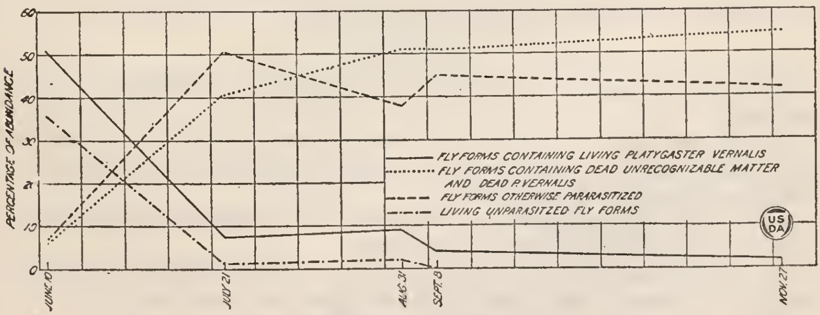

FIG 6.—Mortality of *Platygaster vernalis*, as observed in collections of Hessian fly forms made at Mount Holly Springs, Pa., in 1920. (Table III.)

since the remaining 35.82 per cent could be accounted for by hosts containing dead unrecognizable matter and dead *vernalis*.

In 1919 (Table III), by July 11, 60.03 per cent of the *vernalis* had been destroyed. By October 10 of the same year 93.91 per cent had died. Of these at least 46.14 per cent were killed in competition with other hymenopterous parasites of the Hessian fly.

In 1920 (Table III), by July 21, 85.27 per cent were destroyed, and by the 27th of November the mortality had reached 96.08 per cent. Although other parasites undoubtedly were responsible for the death of many of these, the figures on this date show such a high percentage of puparia containing dead, unrecognizable matter and dead *Platygaster vernalis* that the entire 96.08 per cent could not be assumed as having been killed in competition with other parasites.

In 1921 by the 12th of July (Table III), there was a mortality of *vernalis* to the extent of 78.97 per cent and by September 23 the percentage of death reached 92.11 per cent. While in the results of this last examination the death of nearly all of the *vernalis* could be accounted for by the large number of puparia containing dead unrecognizable matter and dead *P. vernalis*, yet the figures from the collection taken June 21 of the same year show that fully 31.64 per cent had already been killed as early as June by other hymenopterous parasites.

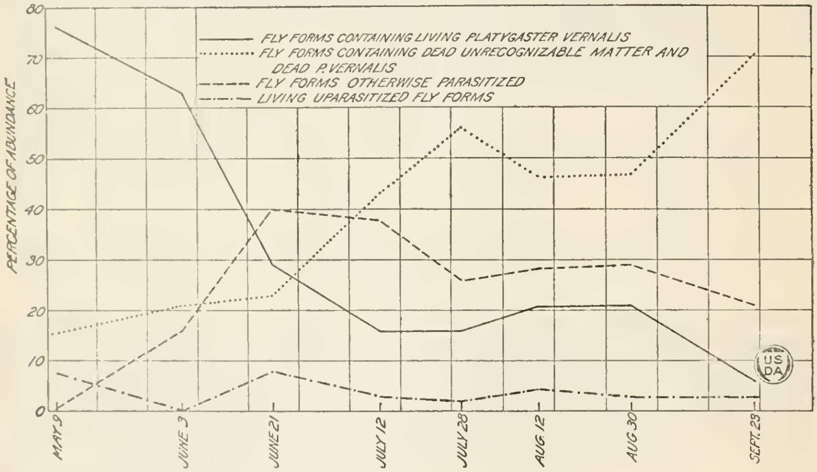

FIG. 7.—Mortality of *Platygaster vernalis* as observed in collections of Hessian fly forms made at New Windsor, Md., in 1921. (Table III.)

SUMMARY

From the foregoing observations the following conclusions may be deduced·

(1) During each year the death rate of *Platygaster vernalis* was very high, being not less than 81.33 per cent for any one year, and in 1920 being as high as 96.08 per cent.

(2) A large percentage of the mortality of *P. vernalis* was due to competition with other Hessian fly parasites.

(3) During the years 1918, 1920, and 1921 for the localities under observation, *P. vernalis* was more effective than all other parasites of the spring generation of the Hessian fly combined.

It should be stated that although the attacks of the other parasites are highly detrimental to the multiplication of *Platygaster vernalis*, yet they supplement the latter sufficiently well to effect a very high death rate of the Hessian fly. They also act as a safeguard in case of scarcity of *vernalis*. In all cases, moreover, the hyperparasitism appears to be entirely accidental. It would therefore be unwise to discount too greatly the value of the other parasites.

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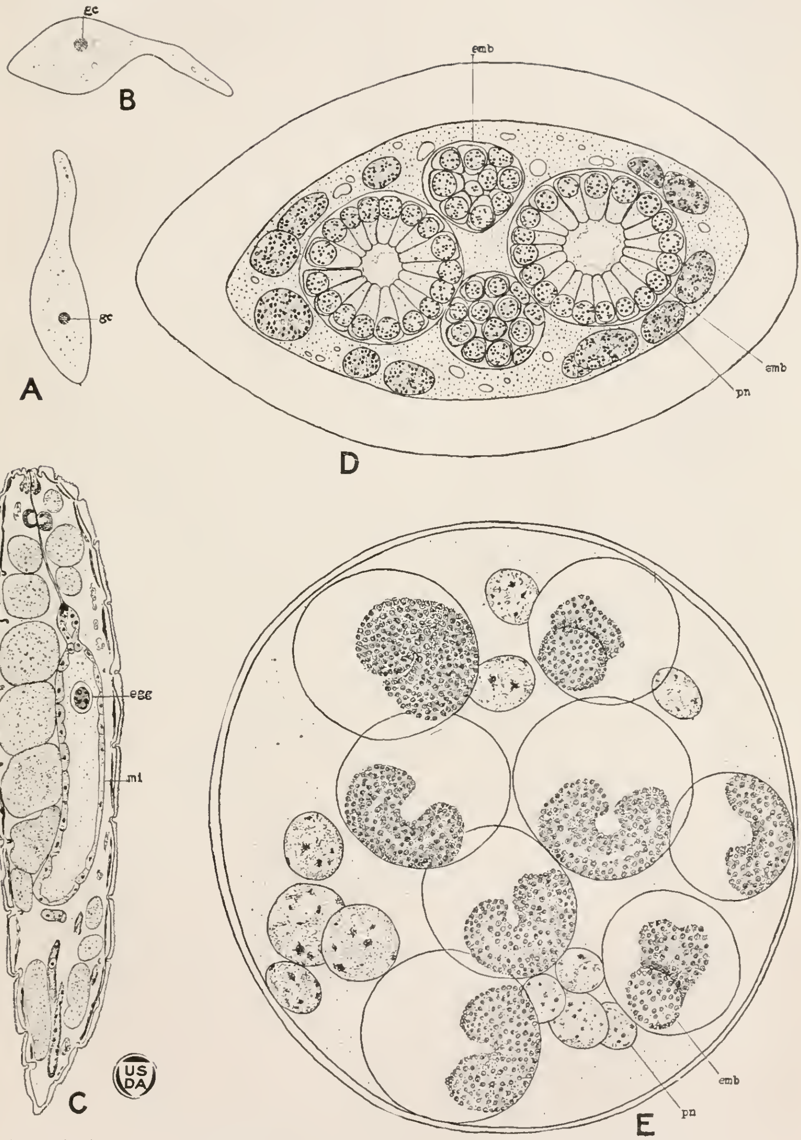
PLATE 1

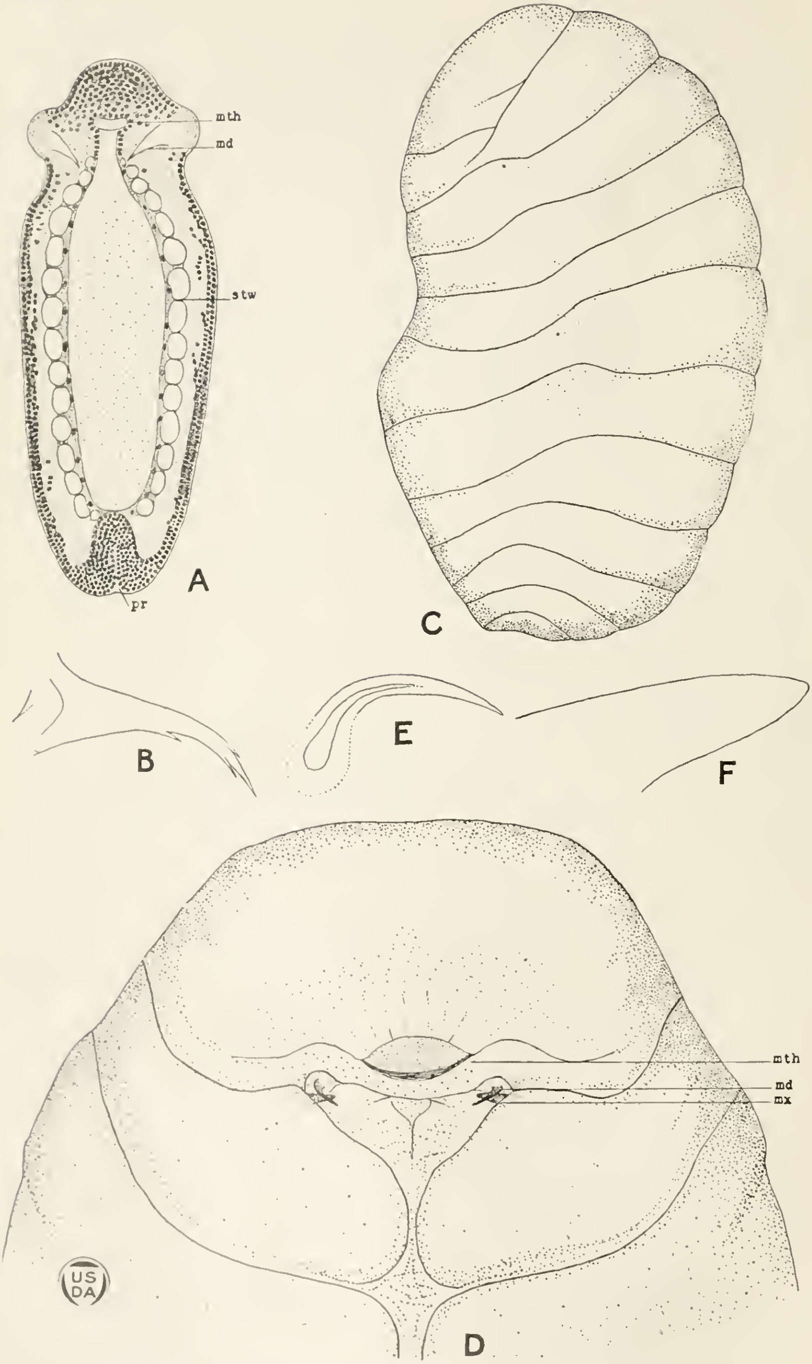
Platygaster vernalis:

A.—Longitudinal section of egg before oviposition. × 397.
B.—Longitudinal section of egg immediately after oviposition. × 391.
C.—Longitudinal section of Hessian fly larva containing a *vernalis* egg within the midintestine. × 29.
D.—Sagittal section of *Platygaster vernalis* egg about 11 days old, showing four embryos in the blastula stage of development and numerous paranuclear masses present. × 695.
E.—Parasite mass of *Platygaster vernalis* containing eight embryos at an advanced stage of development. × 87.

Explanation of symbols on Plates 1–4

egg=egg.
emb=embryo.
gc=germ cell.
go=gorgeret.
md=mandible.
mi=midintestine.
mth=mouth.
mx=maxilla.
pn=paranuclear mass.
pr=proctodaeum.
sh=sheath.
ssp=sternal spatula.
stw=stomach wall.
styl=stylet.


gc
B
gc
A
emb
emb
pn
D
egg
mi
C
emb
pn
E


mth
md
stw
pr
A
C
B
E
F
mth
md
mx
D

3

42

PLATE 2

Platygaster vernalis:

A.—Ventral view of primary larva, showing certain outstanding features of the internal anatomy, such as the stomach wall and proctodaeum. × 132.

B.—Mandible of primary larva. × 417.

C.—Lateral aspect of mature larva. × 77.

D.—Ventral aspect of head and thorax of mature larva, showing mouth and mouth parts. × 212.

E.—Mandible of mature larva. × 833.

F.—Maxilla of mature larva. × 833.

PLATE 3

Platygaster vernalis:

A.—*Platygaster vernalis* cocoons from a single Hessian fly puparium. × 20.
B.—Lateral aspect of *P. vernalis* pupa. × 96.
C.—Ovipositor of *P. vernalis.* × 347.
D.—Parts of ovipositor of *P. vernalis*, showing sheath, gorgeret, and stylet.
E.—Ovary of *P. vernalis.* × 346.
F.—Exit holes in Hessian fly puparium made by adult *P. vernalis* in emerging. × 13.

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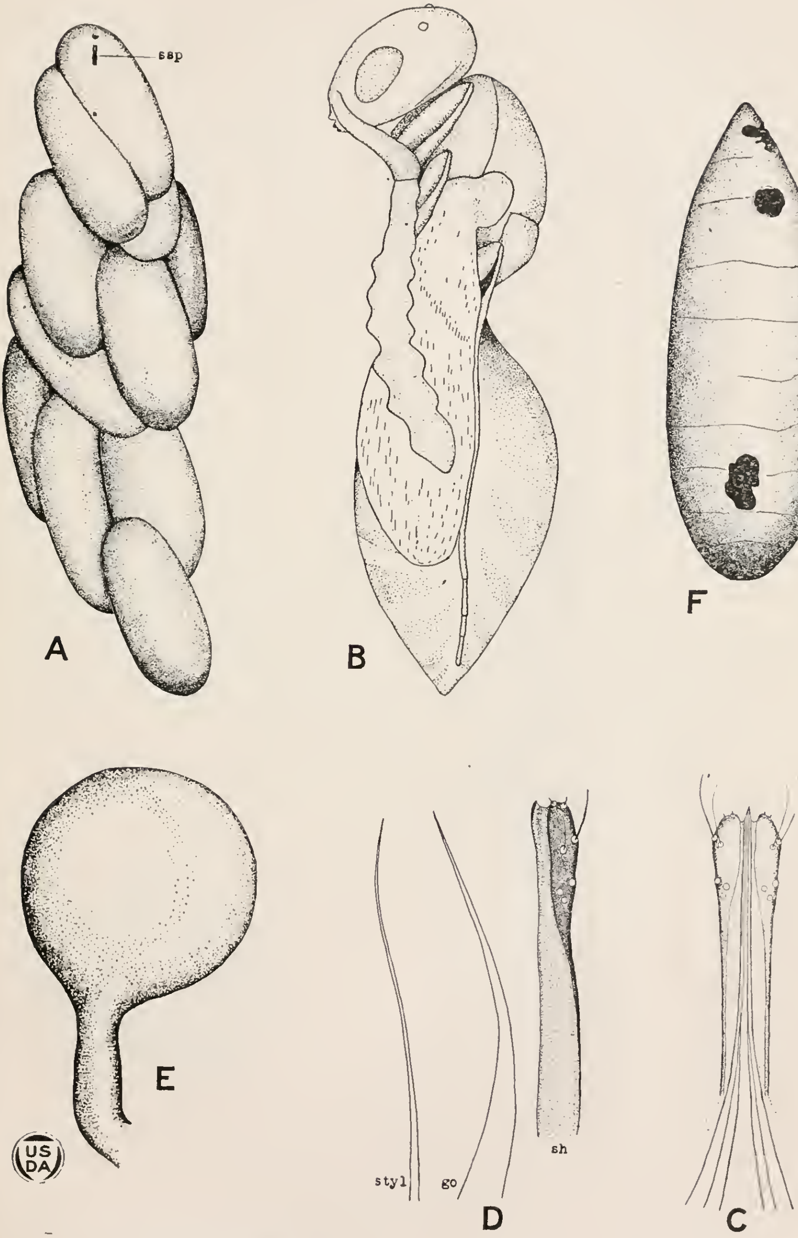

Platygaster vernalis, Hessian Fly Parasite PLATE 4

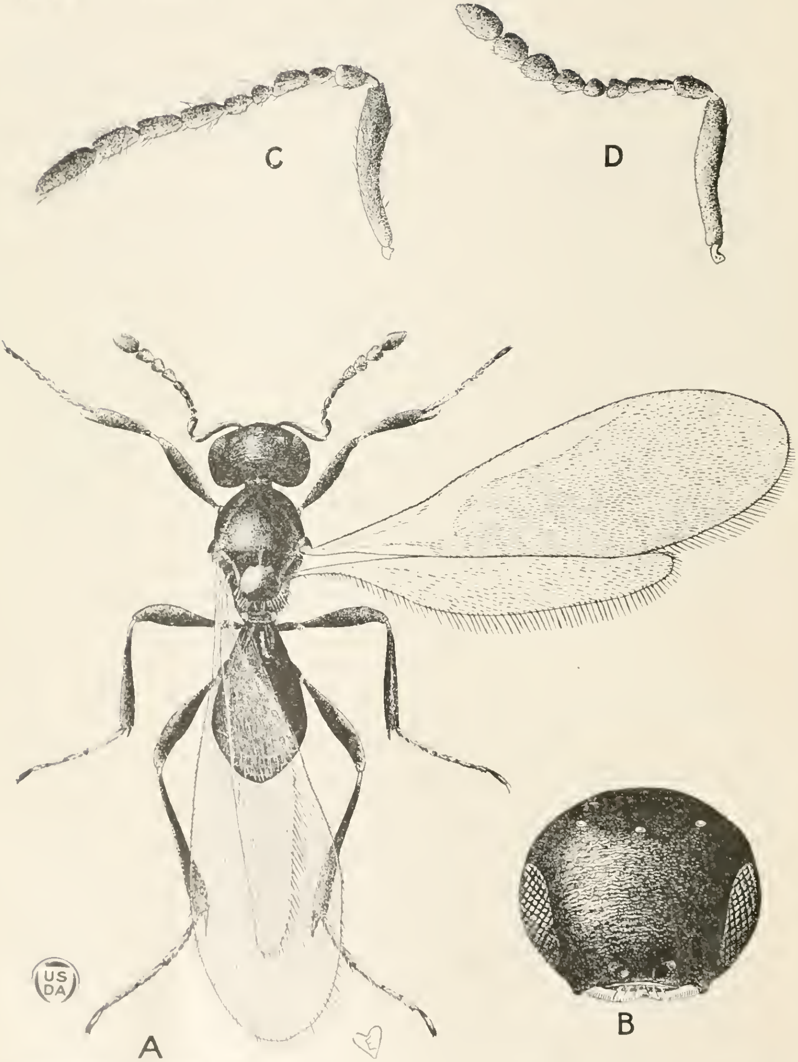

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PLATE 4

Platygaster vernalis:

A.—Adult female.
B.—Frontal view of head of adult.
C.—Antenna of male.
D.—Antenna of female.

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TRANSMISSION, VARIATION, AND CONTROL OF CERTAIN DEGENERATION DISEASES OF IRISH POTATOES[1]

By E. S. SCHULTZ, *Pathologist, Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*, and DONALD FOLSOM, *Plant Pathologist, Maine Agricultural Experiment Station*

INTRODUCTION

Progress in solving the well-known problem of degeneration in the Irish potato, *Solanum tuberosum L.*, has been comparatively rapid during the last decade. With this progress the apparent complexity of the problem has increased. Consequently the results of many investigators are needed and frequent reports from the various workers in this field are desirable.

Of the many phases of the problem in question, the writers have restricted their efforts largely to those of the transmission, variation, and control of certain diseases causing degeneration. This paper both confirms the results of workers in other regions and also discloses hitherto unreported principles that must be respected if control is to be attained ultimately.

TERMS AND TECHNIC USED IN THESE STUDIES

As pointed out by Quanjer (*39, p. 127*),[2] it is desirable that those working with degeneration diseases agree as to the meaning of terms employed. As the same author also points out in referring to the English use of the term "leaf curl," this agreement has not been realized. The varietal modification of symptoms described by Quanjer (*39, p. 130*), by Murphy (*29, p. 34*), and by the writers in this paper, makes it difficult to reach such an agreement in the use of terms until at least the same variety is used by different investigators for a study of the various diseases. Under this state of affairs it seems necessary first to define the terms to be used in this paper. Certain general methods of technic also will be described here to obviate repetition.

Degeneration diseases of potato are here considered to be those transmissible or infectious diseases which are perpetuated indefinitely by vegetative growth and propagation, and of which no cause, either organic or inorganic, has been identified and demonstrated. They include maladies of which the etiology is not fully understood. Although intracellular

[1] Accepted for publication May 2, 1923. This paper is based upon investigations carried on as a cooperative project between the Office of Cotton, Truck, and Forage Crop Disease Investigations of the Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Plant Pathology of the Maine Agricultural Experiment Station. Unless otherwise indicated, the work was performed in northeastern Maine in the vicinity of Presque Isle. The order of arrangement of the authors' names is not intended to indicate that one cooperating institution contributed more than the other to the results.

[2] Reference is made by number (italic) to "Literature cited," p. 115-117.

amoeboid bodies have been found associated with mosaic of tobacco (*18*, *34*), with mosaic of sugar cane (*26*), with mosaic of corn (*22*), with mosaic of *Hippeastrum equestre* (*23*), with mosaic of *Hippeastrum johnsonii* (*25*), and with rosette disease of wheat (*24*), nevertheless, the causal relation of these bodies and organisms to those diseases remains to be demonstrated. For convenience this unknown cause, which seems to be associated with the plant juice, will be referred to here, as elsewhere, as a contagium or a virus. Degeneration diseases are further characterized generally by dwarfing and chlorosis, and by the absence of the first symptoms from foliage that has attained complete growth before the introduction of the virus.

In the absence of definite information on the exact causes of degeneration diseases of plants, it is necessary to define such diseases, both as a class and as individuals, entirely in terms of behavior and symptoms. A number of characteristics are considered by the writers and others as being elementary "unit symptoms" of degeneration diseases of the potato. Dwarfing consists essentially of reduction in size of parts (Pl. 2, A, 2) rather than in their number, although both may occur together. The supporting parts of the shoots and leaves are reduced in length and thickness. The leaf blades are reduced in area, possibly only apparently so sometimes when wrinkling or ruffling is present. Spindliness is lateral dwarfing, resulting in stems or tubers being abnormally slender (Pl. 8, A, 2, C, 1, 2). Chlorosis is a yellowing or paleness that affects the leaf blades and is assumed as being diffused unless designated as mottling. Mottling is a localized chlorosis consisting of spotting of the leaf blades by light green areas, which may or may not occur in contact with the larger veins, and which vary in shape and degree of paleness. These discolored spots are punctate, elongate, circular, angular, and irregular. They vary from a barely discernible fading of the green to an almost pure yellow, often in the same spot. They seldom exceed a few millimeters in any dimension, and their distinctness of outline differs, usually in proportion to the degree of discoloration. They are more readily seen in diffused light than in direct sunlight. Wrinkling is an abnormal unevenness of the leaf-blade surface due to depressions and prominences not arranged in any uniform manner as with rugosity (Pl. 1, A, 1, 2). Rugosity differs from wrinkling in having depressions only at the veins and in having the prominences of uniform height (Pl. 3, C; 4, A, 1). Ruffling is an abnormal unevenness of the leaf-blade surface caused by ridges that develop or become more pronounced with passage from the midrib to the lateral margins, resulting in waviness of the margin (Pl. 1, A, 1, 2). Curling is an abnormal bending of the leaf blade downward along the main vein (Pl. 3, A). Rolling is an upward curving of the sides of each leaflet with the midrib at the bottom of the trough thus formed (Pl. 6, C, 2). Uprightness may appear in both leaves and stems but is assumed to be in the latter alone unless otherwise stated (Pl. 10, B, 2, C, center). It is characteristic of normal plants in most varieties when small and probably in diseased plants is often due to dwarfing. Rigidity may affect either stems or petioles, or both; brittleness may do the same (Pl. 6, A, 2, B, 1). Necrosis is the premature death of tissues accompanied and manifested by their turning brown. It may appear as spot necrosis or spotting (Pl. 5, B), with brown flecks of leaf blade usually more conspicuous on the upper surface; or, as streak necrosis or streaking, with brown streaks first, most evident on the lower surface of the

leaf veins (Pl. 5, B) but sometimes also apparent on the upper leaf surface, stems, and petioles; or, it may appear as marginal necrosis or burning, beginning at the margin and progressing inward. Streaking may spread out into the parenchyma as spotting or burning. Leaf dropping concerns entire leaves, usually the lowest first, and then progressively higher ones. It may begin with a collapse of the green petioles, followed by wilting and finally by necrosis of the petioles and blades. Premature death usually is preceded by leaf dropping and necrosis and finally involves the whole stem and shoot.

The preceding is concerned with isolated unit symptoms. Certain frequently occurring groupings of these unit symptoms have been considered as indicating the presence of certain viruses and have been designated by various descriptive terms such as mosaic (*32, 45*), mosaic dwarf (*21; 32, fig. 29*), curly dwarf (*32, 38*), streak (*5, p. 50; 33*), leaf roll (*32, 41*), and others. These symptom-complexes will be considered later in this paper, except that it may be stated here that a degeneration disease is considered to be of the mosaic type if there is mottling and abnormal unevenness of the leaf surface.

The writers have performed inoculation, or the introduction of material (inoculum) from a diseased plant into a healthy one, by means of several methods. Tuber grafts have been made by keeping in contact the cut surfaces of split tubers. Stalk grafts have been made by splitting the healthy stalk and inserting the wedge-shaped base of a diseased scion and fastening with cord and water-impermeable tape. Leaf-mutilation inoculation consists of bruising the healthy leaves with the fingers or palms and applying to the spongy mass of mutilated leaf tissue juice that has been expressed from a diseased plant. This may be repeated at intervals of several days. Capillary-tube inoculation consists of inserting small glass tubes, containing the inoculum, into the stalk. Aphids have been transferred from the diseased to the healthy plant growing in separate cages or have been both transferred and allowed to disperse with the diseased and healthy plants in the same cage. In the latter case there was root contact, which has also been tried with aphids absent. Natural inoculation is effected by aphids.

A control to an inoculated plant is reliable only when in the same tuber unit; that is, in the group of plants or hills growing from the separated parts (seed pieces or sets) of the same tuber. The members of a tuber unit are sometimes separated and planted in several places or cages. Current-season symptoms are those appearing in the same season in which inoculation occurs, in contrast to those following both inoculation and tuber perpetuation of the disease. These two classes of symptoms, differing only as to the immediate origin of infection, have been designated respectively as primary and secondary by Quanjer (*38, p. 36; 39, p. 130*), and respectively as secondary and primary by Edgerton (*12, p. 7*) following the nomenclature of sugar-cane mosaic. The incubation period is the length of time between inoculation and the appearance of current-season symptoms. If current-season symptoms are absent or if they appear in plants under cages or in other abnormal conditions, it is desirable to observe the second generation following tuber perpetuation. If they appear in normal conditions in a high percentage of the inoculated plants and not in the controls, uncontrolled natural transmission in the field, presumably by insects, will sometimes cause late-season infection of the controls so that observation after tuber perpetuation is of less value than that made before. The second-generation progeny of a hill will be

called a hill lot. Hill lots from the same tuber unit are the beginning of a tuber-unit strain. A hill lot usually is planted so that it consists of consecutive undivided tuber units, in order that comparison between tubers may be made more easily.

TRANSMISSION AND DIAGNOSIS IN THE GREEN MOUNTAIN VARIETY

A given degeneration disease often differs with the variety of potatoes, so that the data first presented will be limited to the variety with which the writers are most familiar. In this variety, the Green Mountain, several diseases have been distinguished, occurring both singly and in combinations. They will be considered first separately and then comparatively, with final consideration of the question of combinations.

MILD MOSAIC

Mosaic of potatoes was first described in the literature by W. A. Orton (*32, p. 42*; *29, p. 59*) although Quanjer claims to be the first to distinguish it (*39, p. 128*):

> Gradually I learned to distinguish the leaf-roll type, and another type which reminded me of the mosaic disease of tobacco ...... Afterwards Danish and American investigators also began to distinguish mosaic.

The term "mosaic" will be used here as covering several types of diseases all characterized in common by mottling and wrinkling. The term "mild mosaic" has been used by Tolaas (*49, p. 10*) and is now used by the writers as equivalent to the slight, slight plus, and, in part at least, the medium stages of mosaic as previously described by them (*40, p. 316*). The characteristic symptoms consist of slight dwarfing, distinct mottling, wrinkling, and some ruffling (Pl. 1, B, 1, C,2). As will be shown later, it is more easily transmitted than leaf roll and is less easily transmitted than rugose mosaic and streak. The tuber symptoms are a general average reduction in size.

CONTACT INOCULATIONS

As reported previously (*40, p. 319*), tuber-graft inoculation may result in infection with current-season symptoms. Stalk grafts may do the same (*45, p. 251–53; 40, p. 319*). Temporary contact of healthy and mosaic seed pieces all cut with the same knife did not cause appreciable infection (*40, p. 332–33*). Contact of uncut tubers in storage probably results in no transmission. Several stocks with a small percentage of mosaic have been used to furnish seed for greenhouse experiments early in the winter and also for field experiments after winter storage, but did not have a higher percentage of incidence in the field after the longer period of contact of the tubers in storage.

Contact of healthy and mosaic shoots was not followed by infection in greenhouse experiments (*45, p. 264–65*) except after aphids had fed upon these shoots.

Contact of both shoots and roots in the greenhouse (*40, p. 333*) was not followed, during three months of active growth subsequent to establishment of contact, by any mosaic symptoms. This was in marked contrast with results in a contemporary experiment wherein plants that started to grow 2 weeks later and that were harvested at the same

time showed symptoms from 4 to 10 weeks before harvest as the effect of aphid transmission.

Complete contact inoculation of 10 hills inside of 5 field cages in 1919 was accompanied by transmission of mosaic in the first generation only in one cage where aphids accidentally gained access (*40, p. 333–34*).

A greenhouse experiment was performed in the winter of 1919–20 with aphids excluded by insect cages and with two healthy plants growing in the same pots with mosaic ones. The tubers when dug remained attached to the rhizomes and those of the healthy plants produced healthy progeny.

In field insect cages in 1920, healthy hills grown with roots, shoots, or both in contact with mosaic hills (Table I) displayed no symptoms and had healthy progeny, except when aphids were present.

TABLE I.—*Mild mosaic inoculations of caged Green Mountains in 1920 (Presque Isle, Me.)*

Inoculation.			Progeny, 1921.	
Series.	Method.	Hills.	Tuber units.	Mosaic.
				Per cent.
1	Aphids, flea beetles, and full contact (of roots and shoots)	[a] 5	12	83
2	Flea beetles and full contact	[a] 1	8	0
3	Full contact (controls to Series 1, 2)	[a] 6	20	0
4	Root contact	[a] 12	47	0
5	Flea beetles and shoot contact	[b] 6	24	0
6	Flea beetles	[b] 6	26	0
7	Shoot contact	[b] 6	18	0

[a] Six 4-hill tuber units each represented in Series 1 or 2, 3, and 4.
[b] Six 3-hill tuber units each equally represented in Series 5, 6, and 7.

During the winter of 1921 and 1922, in a greenhouse experiment at Washington, D. C., 15 healthy tubers were split; one half of each tuber was planted in an 8-inch pot not in contact with a mosaic plant, the other half was planted in a 10-inch pot with a tuber half taken from a mild mosaic potato vine. Ten of the healthy half tubers were planted in contact with the mosaic seed pieces but not grafted; the remaining 5 were planted 6 inches from the mosaic seed pieces. Nine of the mosaic seed pieces represented the Bliss Triumph variety, while the remaining six mosaic tuber halves represented the Green Mountain variety.

The fifteen 10-inch pots were kept under aphid-proof cages from December 30, 1921, when the tubers were planted, until April 11, 1922, when they were harvested. The fifteen 8-inch control pots with the single healthy half-tuber seed pieces were kept uncaged in the same greenhouse as the caged lots. Three examinations for aphids during the course of this experiment did not disclose a single aphid within the cages. These observations also revealed that every vine from the 15 mosaic seed pieces showed distinct mild mosaic mottling, and that the vines from the 15 healthy seed pieces remained free from mosaic mottling until they were harvested April 11, 1922, when the majority of the plants showed distinct signs of maturity. The second-generation plants from the healthy seed pieces like the first generation also failed to show any signs of mosaic mottling, while under the same conditions distinct

mosaic mottling appeared on the vines from mosaic seed pieces. This test confirms earlier, well-controlled experiments, showing that root and vine contact alone do not result in mosaic transmission.

Further data on contact inoculation will be given when considering intervarietal transmission.

JUICE-TRANSFER INOCULATIONS

Aphids are a natural means of transmission and when of cosmopolitan species like potato aphids, *Macrosiphum solanifolii* Ashm., or spinach aphids, *Myzus persicae* Sulz., are usually readily available and capable of being multiplied to sufficient numbers under control conditions, at least for a limited number of experiments. The difficulty with aphids is chiefly one of supplying the proper control conditions for inoculations on a large number of plants in the open field. Aphids also cause uncertainty regarding the interpretation of the presence or absence of certain symptoms, inasmuch as they often produce dwarfing, mottling, chlorosis, wrinkling, streaking, and complete necrosis directly even when nonvirulent, especially if abundant, and as, on the other hand, they may be influenced by certain conditions so that they do not accept a new host readily. Therefore an artificial method of transmission if effective may be preferable to the use of aphids, because of greater ease of application to a large number of plants, because of the absence of direct inoculation injury on the new growth, and because of the greater uniformity of treatment. It also introduces no disturbing factor into an aphid-free field or greenhouse as would be done by the introduction of uncaged aphids or of aphid cages. It sometimes is necessary when aphids fail to increase to sufficient numbers.

While each method has advantages, both should be used, especially with such virus diseases as have been transmitted experimentally with neither or with only one. As an artificial method of inoculation, tuber grafting is handicapped by the uncertainty as to how many new diseases were acquired by the parent vines while apparently healthy, while stem grafts in the open field often fail because of hot, dry weather. As a result, the leaf-mutilation method (p. 45) has been used extensively by the writers.

It has been reported (*45, p. 253–54; 40, p. 320–26*) that mosaic can be transmitted by leaf-mutilation inoculation, but there is some uncertainty as to what types of mosaic were involved. The type diagnosed as mild mosaic has since been used.

Such inoculations in 1921, in the open field, and during the preceding winter, were intervarietal and will be described later. Some were performed and repeated inside of insect cages within the Green Mountain variety, upon 6 hills in as many tuber units. Two hills showed current-season symptoms and their progeny, three and four tuber units, respectively, were mild mosaic. The other 4 inoculated hills and 18 other caged hills in the same tuber units, were all healthy in both generations, originating from healthy control hills caged in 1920. Six of the 18 other hills were inoculated with the capillary-tube method (p. 45) and 12 were controls.

On December 19, 1921, in the greenhouse at Washington, D. C., eight potato plants, from 3 to 8 cm. in height, were treated with juice from a mild mosaic vine by leaf-mutilation inoculation. Four of these plants were then kept in a moist chamber for 24 hours while the remainder were outside of a moist chamber. By the end of four weeks, mild

mosaic appeared on the top leaves on one of the plants. At this time the vines varied from 30 to 40 cm. in height. On the vines in the second generation, however, this and two additional cases developed mild mosaic. These three mottled vines—that is, 37 per cent of the treated plants—included all the plants, except one, kept in a moist chamber for 24 hours after treatment. None of the inoculations outside of moist chambers were effective. The three successful inoculations were performed on three of the four plants that were 5 cm. or more high when inoculated.

On December 21, 1921, eight healthy plants were treated as those inoculated on December 19 had been, with the exception that only two of the plants were kept in moist chambers until 24 hours after inoculation. One of these plants developed mild mosaic before harvest, and in the second generation the other showed mild mosaic. Hence only the two vines which were placed in moist chambers developed mosaic. In each of the foregoing series only a single inoculation was performed.

Leaf-mutilation inoculation (not repeated) with mild mosaic had no effects when made in the Orono (Me.) greenhouse in the winter of 1921–22, in 10 hills from five tuber units. The 10 progeny of the sources of inoculum and the 27 progeny of the inoculated hills were all grown in the same greenhouse in the following summer, when neither group showed any mosaic symptoms. If infection took place, it was masked in the second generation. This was in contrast to parallel inoculations with the rugose type of mosaic, where symptoms appeared in both generations (p. 52).

Fifteen healthy plants representing six different tubers were inoculated four times at approximately weekly intervals, with inoculum taken from a mild mosaic vine and applied by the leaf-mutilation method. The first inoculation was made when the plants were from 3 to 9 cm. in height, February 1, 1922, and the last on February 23, in the Washington (D. C.) greenhouse. On March 1 the first mild mosaic symptoms appeared on the young leaves in 2 plants and by March 21, 10 additional plants, or 80 per cent, showed mottling. The same number of vines were mild mosaic on all leaves in the second generation. The character of these symptoms was like those on the vines with a single inoculation with mild mosaic made in December, previously described. Accordingly, repeated inoculations produced a higher percentage of infected plants than a single inoculation. All uninoculated controls from the same tubers as the inoculated plants remained healthy in both generations. It may be pointed out that the three unsuccessful inoculations and one with incomplete current-season symptoms (on only one shoot) were made on plants among the 7 which were 5 cm. or less in height at the time of the first inoculation.

Leaf-mutilation inoculations with mild mosaic were performed again in 1922, both in the open field and inside of insect cages, with current-season symptoms in the cages (Pl. 2, A, 2). (For details see later section on "Inoculations performed in 1922.")

These results show that leaf-mutilation inoculation is sometimes, but not necessarily, an effective means of infection with mild mosaic and might explain the skepticism of certain workers in Holland regarding this method were it not that this skepticism is apparently not based on any trial of this method, but rather on a needle-prick method (*4, p. 19*), which the writers long ago discarded as useless (discussion on streak, p. 53).

INSECT INOCULATIONS

It is thought that mild mosaic alone was involved with several experiments previously reported (*45, p. 261–66; 40, p. 326–28; 41, p. 54–55*) as giving transmission with aphids within Green Mountains, but further experiments were made.

In 1921, six healthy hills of as many tuber units were caged, each cage containing two of these hills separated by a hill having both mild mosaic and the spindling-tuber disease. Potato aphids, *Macrosiphum solanifolii* Ashmead,[3] were introduced upon the diseased plants and from each of these either dispersed or were transferred to the healthy hill or hills in the same cage. Later they were sprayed with nicotine solution. Data are presented in Table II.

Two of the five inoculated hills became mosaic, with symptoms only in the progeny. The five control hills in the same tuber units but in different cages were healthy and their progeny, 15 tuber units, were healthy. It is to be noted that complete mosaic infection occurred only in the hill (No. 1 of cage B–3) where there was the earliest dispersal of aphids, where the aphids were not all killed by the first spray application, and where there was only one healthy hill to which to disperse.

In 1921, in another cage, aphids transmitted both mild mosaic and spindling tuber (Table XVI, inoculation No. 14).

TABLE II.—*Mild mosaic and spindling-tuber inoculations of caged Green Mountains with aphids, in 1921*

Cage.	Diseased hill.		Healthy hills.					
	Number.	Aphids introduced.	Number.	Aphids transferred.	Date of spraying for aphids.	Progeny. Total tuber-units.	Progeny. Mosaic.	Spindling-tuber.
							Per cent.	Per cent.
B–1	2	June 28	1	July 9 and 13	July 18	4	0	0
		July 9	3	do	do	4	25	0
B–2	2	June 28	1	July 9 and 18	July 23	2	0	100
		July 9 and July 13.	3	do	do	4	0	75
B–3	2	June 24	1	July 13 (dispersal by July 9).	July 18 and Aug. 22.	5	100	0
			3	Hill removed				

Further data on aphids will be given in connection with interspecific transmission.

Negative current-season results have been reported from transferring flea beetles, *Epitrix cucumeris* Harris, in abnormally large numbers from mosaic to healthy plants (*40, p. 329*). The progeny of the healthy plants were all healthy, whereas 65 per cent of corresponding ones treated with aphids were mosaic.

In 1920, flea beetles again were introduced from mosaic plants into field insect cages in large numbers and allowed to feed upon healthy

[3] The authors wish to thank Dr. Edith M. Patch of the Maine Agricultural Experiment Station and Dr A. C. Baker of the Bureau of Entomology, U. S. Dept. of Agriculture, for frequent identifications of aphids

plants. They also fed upon mosaic and healthy plants in the same cage. In some cages all plants were growing in the field soil, with roots and vines in contact, and in others the healthy plants were potted with only the vines in contact with the diseased plants. Controls with no flea beetles present were grown. In spite of special precautions, potato aphids were introduced with the flea beetles into some cages. The exclusion of aphids is a difficulty also experienced by others (*39, p. 134*). The data have been presented in Table I. No current-season symptoms were manifested. The second generation had mosaic only in the progeny of the hills inoculated by aphids.

In this experiment (Table I, Series 4) attempts were made to test the possibility of transmission by larvae of flea beetles. A healthy Green Mountain plant was caged and outside its cage a mosaic plant was grown partly caged, and subjected under the small cage to severe infestation by flea beetles. The infested part was badly damaged but no flea beetles emerged at any time outside the large cage, whereas introduction of large numbers of this species into other large cages was followed later in the season by the emerging of a second generation numerous enough to skeletonize the laves. It seemed that the flea-beetle larvae feeding upon the mosaic plant did not travel far enough to reach the next hill. No effects were evident in either generation.

In 1919, experiments were performed with Colorado potato beetles, *Leptinotarsa decemlineata* Say., as with the flea beetles, with similar negative results in both the first (*40, p. 329*) and the second generations. This beetle does not move from plant to plant as much as does the flea beetle. Its feeding habits result in more complete destruction and less wounding of the tissues. Although larger, its individuals are less numerous in the early part of the season when the plants are small. Altogether, it would seem to be less liable to prove a carrier than the flea beetle.

LEAF-ROLLING MOSAIC

The symptoms of "crinkle" as described by Murphy (*29, p. 71–74*) seem to be applicable in part to a leaf-rolling type of mosaic "approaching somewhat to the appearance of curly dwarf," and in part to "rugose mosaic" as described later in this paper. The writers here use the term "leaf-rolling mosaic" as designating a symptom complex that so far has been irreducible to simpler complexes, and that consists of slight dwarfing, diffused mottling, wrinkling, slight ruffling, and rolling of the upper leaves (Pl. 2, B, 2, C, 1). It is different from mild mosaic in respect to the distinctness of the mottling, the presence of rolling, and the effects in combination with the spindling-tuber disease, and is similar to it in infectiousness. The tuber symptoms are a general average reduction in size. It is distinct from leaf roll.

Intervarietal inoculations of Green Mountains will be described later. Leaf-mutilation inoculations of 10 hills within the variety in the open field in 1922 gave no current-season symptoms, in contrast with rugose mosaic. Inoculations of an apparent combination of leaf-rolling mosaic and the spindling-tuber disease were made within the Green Mountain variety in 1921 in an insect cage. Potato aphids from a "curly dwarf, vaguely mottled" hill dispersed and were transferred to a healthy hill whose progeny were curly dwarf in two tuber units and spindling tuber in both these two and in the third tuber unit which was not curly dwarf. The progeny of the diseased hill which was the source of the inoculum,

and of the inoculated hill, are shown in Plate 3, A, B. It seems probable that the spindling-tuber disease (p. 55) was introduced into all three tubers of the inoculated hill, while leaf-rolling mosaic was introduced into only two, both diseases being manifested when in combination as mottled curly-dwarf (*47, Pl. 2; 32, Pl. 13*).

Intervarietal inoculations, described later, transmitted leaf-rolling mosaic alone to Green Mountains (Pl. 2, C 1).

RUGOSE MOSAIC

A type of mosaic much more infectious than mild mosaic, as will be shown later, is further differentiated by distinct dwarfing, more chlorosis and more diffused mottling, a more rugose type of wrinkling, and a tendency to show brittleness, spotting, streaking, leaf dropping, and premature death (Pl. 1; 3, C, 1; 4, A, 1), especially when in combination with the spindling-tuber disease. It is equivalent in part to the medium-plus mosaic of the writers' previous publications (*40, p. 316*). The tuber symptoms are a marked reduction in size.

The writers believe that most of Murphy's crinkle (*29, p. 71–74*), is identical with the type here designated as "rugose mosaic" with some leaf-rolling mosaic symptoms included by Murphy. It is probable that the name "crinkle" is more descriptive of this disease in a large number of varieties, because of the mottling being generally masked, but the facts, first, that there is mottling, and, second, that the masking of mosaic is generally realized to be fairly common, make it seem unnecessary to abandon here the well-known term "mosaic." The symptoms of "crinkle" as given by Atanasoff (*4, p. 15–16*) include "a heavy mosaiclike variation in color," which can serve very well to describe the writers' "rugose mosaic." This type of mosaic in some varieties (*42*) and in some combinations (*14*) produces an extreme form known as "mosaic dwarf." "Mosaic dwarf," however, as suggested by Krantz and Bisby (*21, p. 7*), seems to the writers to contain more than one disease or symptom-complex. A current-season or primary symptom of crinkle is leaf dropping (*39, p. 139*), which is also a symptom of other degeneration diseases and combinations. Rugose mosaic is more infectious, or at least more generally conspicuous, than mild mosaic in varieties in the Cobbler, Rural, and Rose groups (*48*) and perhaps others (*45, p. 249*), and also is more common in southern regions than in northern.

It is thought probable that the striking current-season symptoms reported for mosaic leaf-mutilation inoculations in the open field in 1919 (*40, p. 320–23*) were involved with the rugose type of mosaic. Intervarietal transmission to Green Mountains was effected in 1920 and 1921 (p. 66).

Leaf-mutilation inoculations with rugose mosaic were made in the Orono, Me., greenhouse in the winter of 1921–22, in eight hills from four tuber units. The sources of inoculum were three hills that were progeny of field-grown plants with streaking but, in one case, with no mottling, and showed dwarfing, mottling, wrinkling, spotting, burning, and leaf dropping. Of the inoculated hills, two showed leaf dropping in 17 days, five in 24 days, and one in 42 days, and one showed mosaic in the uppermost leaves. Most of the progeny (17 of 18) of the inoculated hills, when grown in the same greenhouse in the following summer, were mosaic, completing the contrast to parallel inoculations with mild mosaic where no symptoms ap-

peared in the inoculated hills and where even the progeny of diseased hills showed no symptoms during the summer (p. 49). The eight controls and 13 of their progeny grown in the greenhouse were healthy.

A number of leaf-mutilation inoculations with rugose mosaic were performed again in 1922, usually with current-season symptoms in the open field as well as in the cages (p. 76).

Intervarietal aphid transmission of rugose mosaic to Green Mountains will be described later (Pl. 4, A).

STREAK

Most of the streaking seen by the writers has been associated with rugose mosaic (Pl. 4, B, C), but in at least one case the latter was absent so that streak (*5, p. 50; 33; 29, p. 76–82; 4*) may be considered as a distinct symptom complex. The current-season symptoms appear in a certain order after leaf-mutilation inoculation, as streaking and spotting, burning, brittleness, leaf dropping, and premature death, with no mottling except for chlorotic spots a few hours previously in the places where streaking and spotting occur, and no wrinkling (Pl. 5, A, C; 11, C; 12,B).

Symptoms following tuber perpetuation are extreme dwarfing, wrinkling, curling, rugosity, brittleness, leaf dropping, and premature death (Pl. 6, A, 2, B, 1). In this connection a study of other works (*29, p. 80; 4, p. 16*) is interesting. The tuber symptoms are extreme reduction in size, with some darkening, near the eyes, that resembles small initial infection spots of late blight; *Phytopthora infestans* de By. Cracking or splitting of the tubers has been observed for streak by Atanasoff (*4, p. 9*), as also for curly dwarf and leaf roll by Orton (*32, Pl. 13*), for yellow dwarf by Barrus and Chupp (7), and for unmottled curly dwarf by the writers (p. 60).

Intervarietal transmission was effected in 1921 (p. 69; Pl. 5, A, C) as previously reported (*42*). These results were confirmed with the same methods of inoculation within the Green Mountain variety in 1922, described later. In regard to the 1921 report, Atanasoff (*4, p. 18–19*) writes:

b. Plant juice. Schulz and Folsom claim to have been able to infect potato plants with stipple-streak by means of juice from an infected plant. "In 1921," they write, "juice from a streak plant applied to 20 mutilated Green Mountain and Irish Cobbler plants caused infection in 19, with typical symptoms appearing in some in 12 days.

He then describes inoculations made in Holland "in order to verify this statement" and concludes it to be "highly improbable that the plants infected by Schulz and Folsom have become diseased as result of their infection." Some discussion seems necessary here.

The above quotation (*42*) should have been completed as follows:

Sixty control hills in the same tuber units, from quartered tubers, remained healthy.

The following of inoculation by the disease in 95 per cent of the 20 inoculated hills, in two varieties, with the absence of both inoculation and disease in the 60 control hills, is a contrast more convincing, at least to the writers, than reports of successful grafting with all figures omitted (*4, p. 20*), though the writers by no means doubt the latter report. It is a contrast less impressive than with 100 per cent, or with 95 per cent in 2,000 inoculated hills, but is nevertheless significant.

Verification experiments are misleading when different methods are used. The writers' method was that previously reported by them (*40, p. 320–26*) and included severe bruising of the leaves. The method described by Atanasoff (*4, p. 19*) introduced a drop of juice into a split stem, resembling the capillary-tube and split-stem methods also used by the writers (p. 74, 83) in 1921 and discarded because of ineffectiveness.

Even had Atanasoff's method been similar to the writers' and given negative results, it is well to make allowance for climatic factors. Negative results with aphid inoculations of leaf roll by Murphy (*29, p. 52*) did not prevent later proof, and probably would not have been weighed by him more heavily than his negative results with mosaic and insects (*29, p. 62*) had he not been emphasizing the soil-transmission theory advanced by the Dutch workers but later abandoned by them.

After tuber perpetuation, the progeny of the plants inoculated in 1921 were used in inoculations described later in the section on "Inoculations performed in 1922," and the same positive results were obtained, with spotting of the white corollas in addition.

LEAF ROLL AND NET NECROSIS

Leaf roll, as previously described by the writers (*41, 15*), is characterized chiefly by dwarfing, chlorosis, rolling, uprightness, and rigidity; burning also is common (Pl. 6, C, 2). Phloëm necrosis is a microscopic symptom of great value for positive identification (*39, p. 132*). Leaf roll is less easily transmitted than any other degeneration disease considered in this paper (*29, p. 65*). The size of the tubers is reduced. Streaking (from phloëm necrosis) in the tubers (net necrosis) previously reported as a symptom by the writers (*41, p. 60–74*) and by Gilbert (*17*) and Kasai (*20, p. 52, 69*) has been noted again as a symptom in plants grown in 1921 and 1922. This tuber symptom of leaf roll was reported (*41, p. 68*) as being sometimes induced by varietal and other factors, but did not necessitate (*41, p. 73*) long storage as suggested by Quanjer (*39, p. 132*), and was not a constant symptom as he understood it. The observations made in 1921 on net necrosis are given in a later section (p. 64).

Inoculations within the Green Mountain variety have been reported as giving positive results with stalk grafts and aphids (*41, p. 52, 54–55, 57–59*). Intervarietal inoculations like these, and also unsuccessful attempts to transmit leaf roll by leaf-mutilation inoculation, will be described later (p. 63).

In the winter of 1921 and 1922, an experiment on the effect of healthy potato plants grown in contact with tuber and vines diseased with leaf roll (with insects excluded) was conducted at Washington, D. C. This test was conducted in the same manner and at the same time as the experiment on the effect of growing healthy potato plants in contact with mosaic plants. In this experiment nine healthy half tubers were planted in contact but not grafted with leaf-roll half tubers, while each of the remaining six healthy half tubers was planted 6 inches apart from the leaf-roll half tuber in the same 10-inch pot. Three observations on the vines during the course of the experiment revealed that all vines from leaf-roll tubers showed leaf roll, while the vines from healthy tubers remained free from leaf roll. Second-generation plants from the healthy tubers likewise were free from leaf roll, as microscopic as well as macroscopic examinations disclosed.

SPINDLING-TUBER DISEASE

The spindling-tuber disease (*43, 44*) is characterized always by spindliness and uprightness, and often by a darker green color and slight rugosity. The tubers are abnormally spindling, spindle shaped, cylindrical, and supplied with conspicuous eyes, these symptoms varying somewhat with the variety (Pl. 7, A, 2, 3, B, 1, C, 1; 8, A, 2, B, 2, 3, C, 1, 2; 9, A, 1, B, 1, 2; 10, B, 2, C, in center). A lighter red color obtains in spindling-tuber Red Bliss Triumph tubers than in the healthy tubers of this variety. This disease is readily distinguished from the mosaic diseases by the absence of mottling, and from leaf roll and streak. It is about as easily transmitted as mild mosaic and leaf-rolling mosaic, and therefore less easily than rugose mosaic and streak, and more easily than leaf roll. It reduces the amount of tuber progeny, but because of the difference in shape it may not be readily apparent that the size is reduced below that of uninfected plants.

The spindling-tuber disease of the potato has been recognized for many years by growers and others by various names, such as "running out," "running long," "off shape," "poor shape," "reversion" and "senility." Observations made by the writers on this potato trouble from 1917 to 1921, in connection with investigations on mosaic and leaf roll, indicated its infectious nature. If strains free from this malady were planted near stock with a high percentage of "run out" tubers, a considerable percentage of such tubers resulted in a few years. Elimination of "run out" tubers by selecting only the good, normal-shaped tubers at the time of planting did not produce stock free from such progeny. In fact, in some lots the percentage of spindling tubers increased during this period so that a few lots produced practically 100 per cent spindling tubers. Similar selection of well-shaped tubers by growers from stock with a high percentage of spindling tubers, likewise has failed to produce stock free from this malady. A part of a strain planted under soil, temperature, moisture, fertilizer, and other environmental conditions similar to those of another part, with the exception that it was grown nearer spindling-tuber lots than the other, the next year contained a higher percentage of spindling-tubers. Furthermore, it was noted that normal and spindling-tuber progeny frequently developed in the same hill lot and even in the same tuber unit, just as obtains with mosaic and leaf roll. This was noted by Stewart (*46, Pl. 10*).

Accordingly, during the season of 1921, additional selections for studying the nature of this malady were made. In a field of Green Mountains having less than 1 per cent of spindling-tuber, two lots of healthy hills were selected, one grown next and the other not next spindling-tuber hills; similar selections were made in two fields of Irish Cobbler having 5 and 15 per cent spindling-tuber hills, respectively. Further selections from Green Mountain and Bliss Triumph lots in experimental plots were made. In 1922, these different lots were planted and the percentage of spindling tubers noted as indicated in Table III.

Table III shows that the percentage of spindling-tuber decreases as the distance from diseased hills increases, and that a higher percentage of infection of healthy hills obtains as the percentage of spindling-tuber increases. These data also suggest that spindling-tuber may be transmitted fully as readily as mild mosaic in conditions apparently as favorable regarding the source of infection.

In field 34, Table XXVIII, the spread from diseased rows was such that samples from the first adjacent healthy row gave progeny with 60 per cent of the hills diseased, while six samples from more distant rows gave progeny with only 10 per cent diseased.

TABLE III.—*Effect of proximity of healthy hills to hills with the spindling-tuber disease*

Field.	Lot.	Variety.	1921			1922 progeny.		
			Spindling tuber in field.	Number of healthy hills and location.		Number of tuber units.	Spindling tuber.	Mosaic.
				Adjacent.	Not adjacent.			
			Per cent.				*Per cent.*	*Per cent.*
A	1	Green Mountain	1	10	....	47	30	[a] 6
	2	.....do	1	....	10	40	0	[a] 2
B	1	Irish Cobbler	15	10	....	42	90	....
	2	.....do	15	....	10	38	71	....
C	1	.....do	5	10	....	47	85	....
	2	.....do	5	....	10	51	63	....
D	([b])	Bliss Triumph	[c] 100	48	....	....	60	29

[a] Bulk stock contained 10 per cent mosaic in 1921 and 17 per cent in 1922.
[b] No spindling tuber in 1920.
[c] Adjacent plot. Also 100 per cent mosaic.

Measurements were made of a number of lots of tubers grown in 1921, using a slide caliper of special construction duplicating one devised by F. A. Krantz, of the Minnesota Agricultural Experiment Station. The longest dimension parallel to the structural axis of the tuber was considered the length, while the greatest and least dimensions perpendicular to the axis were called, respectively, the width and depth. Each lot consisted of tubers taken at random from the top of a barrelful that had been picked up by chance in the field. When each tuber was measured, a record was also made of the presence or absence of spindling-tuber symptoms. The results of measuring several Green Mountain lots are summarized in Tables IV, V, and VI. The first three lots in each table consist of diseased tubers, but they were selected in three successive years, respectively. Their similarity in the correlation tables (Table IV) and in the ratios calculated for L/W (length/width) and W/D (width/depth) in Table V show how persistent the dimensional characteristics are when once the disease has entered the tubers and reduced their width, their similarity being more significant for W/D than for L/W. The fourth and last lots, from two different strains, are both healthy and are much more alike than diseased and healthy parts of the same strain or even of the same 1921 plot, especially in W/D. The fifth and sixth lots, grown in the same 1921 plot, are similar although grown on two types of soil, having about 40 per cent of the tubers diseased, and are midway between healthy and diseased lots in dimensional characteristics. It is clear, therefore, that there is a high correlation between mechanically determined dimensions and diagnosis of the spindling-tuber disease from tuber symptoms, even when this diagnosis was made one or two years previously. Mosaic as well as soil had no apparent

effect here, the third and fourth lots both having had mosaic throughout for several years.

With spread in the field and perpetuation in the tubers demonstrated, infection experiments are of interest. As described in connection with mild mosaic (p. 50 and Table II) and with leaf-rolling mosaic (p. 51), aphids transmitted the spindling-tuber disease in insect cages in 1921 to three hills. The controls, in the same tuber units but in different cages, were healthy in both generations.

Table IV.—*Correlation of width with length and depth in Green Mountains grown in 1921*

Lot No.	Width.	Length (centimeters).											Depth (centimeters).				
		6	7	8	9	10	11	12	13	14	15	16	4	5	6	7	8
CT-28	4	....	[a] 1	....	1	....	....	....	....	....	....	....	2	....	....	....	....
	5	1	3	10	4	8	1	....	1	....	....	....	7	21	....	....	....
	6	....	1	7	10	15	8	10	....	1	....	....	....	33	19	....	....
	7	....	....	....	....	3	1	4	4	3	1	....	....	1	14	1	....
	8	....	....	....	....	....	1	1	....	....	....	....	....	....	....	2	....
	9	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
	10	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
CT-29	4	....	3	1	1	....	....	....	....	....	....	....	5	....	....	....	....
	5	1	1	6	11	10	3	5	....	....	....	....	8	29	....	....	....
	6	....	....	1	7	8	11	7	5	2	....	....	....	32	9	....	....
	7	....	....	....	....	....	6	1	4	5	....	1	....	3	11	3	....
	8	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
	9	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
	10	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
V-6Bs. Spindling tuber	4	....	2	....	....	....	....	....	....	....	....	....	2	....	....	....	....
	5	....	7	19	24	9	....	....	....	....	....	....	14	45	....	....	....
	6	....	1	12	16	19	17	3	2	....	....	....	1	57	12	....	....
	7	....	....	....	2	5	2	3	2	1	....	....	1	4	10	....	....
	8	....	....	....	1	1	....	....	....	....	1	....	....	1	1	1	....
	9	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
	10	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
V-6B. Healthy	4	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
	5	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
	6	1	12	3	2	2	....	....	....	....	....	....	....	19	1	....	....
	7	2	6	13	6	7	4	1	....	....	....	....	....	24	15	....	....
	8	....	2	5	10	6	5	....	....	....	....	....	....	5	21	2	....
	9	....	....	....	1	6	3	3	1	....	....	....	....	1	8	5	....
	10	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
CT-13. Caribou loam	4	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
	5	....	....	1	6	7	4	1	....	....	....	....	5	14	....	....	....
	6	....	2	3	3	9	1	2	3	....	....	....	1	19	3	....	....
	7	....	3	4	10	4	6	2	2	....	....	....	....	15	15	1	....
	8	....	1	3	1	2	7	4	3	....	....	....	....	4	12	5	....
	9	....	....	1	2	....	2	....	....	1	....	....	....	1	3	2	....
	10	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
CT-13. Washburn loam	4	....	....	....	1	....	....	....	....	....	....	....	1	....	....	....	....
	5	....	....	5	4	2	2	1	....	....	....	....	3	11	....	....	....
	6	....	2	3	6	6	8	4	2	1	....	....	....	25	7	....	....
	7	....	3	2	10	9	5	3	....	....	....	....	....	16	15	1	....
	8	....	1	....	2	5	2	5	....	....	....	....	....	2	9	4	....
	9	....	....	....	1	....	2	2	....	....	....	....	....	....	4	1	....
	10	....	....	....	....	....	....	....	....	1	....	....	....	....	....	....	1
MSS-S4	4	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
	5	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
	6	....	2	1	2	....	....	....	....	....	....	....	2	3	....	....	....
	7	....	2	6	9	6	2	....	....	....	....	....	....	12	13	....	....
	8	....	....	4	9	8	8	8	2	1	....	....	....	7	28	5	....
	9	....	....	....	....	2	3	2	1	1	1	....	....	1	3	6	....
	10	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....

[a] Figures show numbers of tubers with dimensions indicated at left and at top.

TABLE V.—*Dimensions of tubers of Green Mountains grown in 1921*

1921 plot No.	Strain.	Description of lot measured.	Number of tubers.	Diseased with spindling tuber.	Ratio of length/width (L/W).[1]	Ratio of width/depth (W/D).[1]
				Per cent.		
CT-28	LK, secured in 1916 as mosaic-free.	Spindling tubers selected in winter of 1919-20.	100	98	1.722±.017	1.121±.007
CT-29	do	Spindling tubers selected in winter of 1920-21.	100	99	1.863±.017	1.122±.008
Y-6B	LK, selected in 1916 as mosaic.	Spindling tubers selected in winter of 1921-22.	149	100	1.658±.011	1.144±.007
Y-6B	do	Healthy tubers selected in winter of 1921-22.	101	0	1.213±.012	1.318±.008
CT-13	LK, selected in 1916 as mosaic-free.	Grown on Caribou loam in 1921.	100	40	1.540±.023	1.242±.011
CT-13	do	Grown on Washburn loam in 1921.	100	37	1.541±.021	1.222±.010
MSS-S4	B		80	0	1.302±.013	1.327±.010

[1] Calculations were performed by the Biology Department, Maine Agricultural Experiment Station.

TABLE VI.—*Comparison of plots of Table V, giving difference between means and probable error of difference;*[1] *also ratio between difference and its probable error*

LENGTH/WIDTH

	CT-29.	Y-6B. Spindling-tuber.	Y-6B. Healthy.	MSS-S4.	CT-13. Washburn loam.
CT-28	.141±.024	.064±.020	.509±.021	.420±.021	
Difference/P. E.	(6:1)	(3:1)	(24:1)	(21:1)	
CT-29		.205±.020	.650±.021	.561±.021	
Difference/P. E.		(10:1)	(31:1)	(27:1)	
Y-6B. Spindling tuber			.445±.016	.356±.017	
Difference/P. E.			(28:1)	(21:1)	
Y-6B. Healthy				.089±.018	
Difference/P. E.				(5:1)	
CT-13. Caribou loam					.001±.031
Difference/P. E.					(1:31)

WIDTH/DEPTH

	CT-29.	Y-6B. Spindling-tuber.	Y-6B. Healthy.	MSS-S4.	CT-13. Washburn loam.
CT-28	.001±.011	.023±.010	.197±.011	.206±.012	
Difference/P. E.	(1:11)	(2:1)	(18:1)	(17:1)	
CT-29		.022±.011	.196±.011	.205±.013	
Difference/P. E.		(2:1)	(18:1)	(16:1)	
Y-6B. Spindling tuber			.174±.011	.183±.012	
Difference/P. E.			(16:1)	(15:1)	
Y-6B. Healthy				.009±.013	
Difference/P. E.				(1:1.5)	
CT-13. Caribou loam					.020±.015
Difference/P. E.					(1.3:1)

[1] Calculated as the square root of the sums of the squares of the probable errors of the means.

Further infection experiments including inoculation with leaf mutilation, tuber and stem grafts, and aphids, were initiated on plants in the greenhouse and in cages in the field, as well as under open field conditions. The procedure in these infection tests was similar to those observed with such operations in connection with the writers' studies on mosaic and leaf roll of potato. The results of these experiments are presented in Table VII.

TABLE VII.—*Spindling-tuber inoculations*

Inoculation series.	Variety.	Inoculation.			Inoculated hills.		Controls.[a]		Remarks.
		Type.	Location.	Date.	Number treated.	Infected.	Number.	Infected.	
						Per cent.		*Per cent.*	
1	Green Mountain ...	Tuber graft..........	Greenhouse, Washington, D. C.	Nov. 21, 1921....	10	90	10	0	Mosaic on two plants.
2	.....do............	.....do............	.....do................	Dec. 22, 1921....	7	100	7	0	Mosaic on one plant.
3	.....do............	.....do............	Presque Isle, Me.........	May, 1922......	18	[b]83	20	0	Mosaic on one-third of inoculated plants, both infected and not infected with spindling tuber.
4	.....do............	Vine graft..........	Presque Isle, Me. (in cages).	June, 1922.....	3	33	3	0	
5	Irish Cobbler and Green Mountain.	Leaf mutilation......	.....do................	July, 1922......	14	57	14	0	
6	Green Mountain....	.....do............	Presque Isle, Me., in open field.	.....do.........	20	30	60	5	
7	.....do............	Aphids............	Presque Isle, Me., in cages.	.....do.........	6	33	6	0	

[a] Controls included half tuber seed piece from same tuber as grafted half tuber.

[b] Shown in Plates 8, C, and 9, A.

The data in Table VII indicate that infection with spindling tuber was obtained with tuber and vine grafts, leaf mutilation, and aphids. In inoculation Series 1 and 2 the reaction of the second-generation progeny is represented, which in part accounts for a higher percentage of successful inoculations than in the remaining series. No doubt the second-generation progeny in Series 5 to 7 will show a higher percentage of infection than the first generation, since with spindling tuber as with mosaic and leaf roll, initial infections contracted late in the development of the plants in the first generation will not produce visible macroscopic symptoms in the same generation. Additional evidence on infection with spindling tuber by aphids is disclosed in Table XVI (inoculation No. 5, 8, 13, and 14) and by means of leaf mutilation in Table XVIII.

UNMOTTLED CURLY DWARF

Curly dwarf, described by Orton (*32, p. 37–40*), seems to be in part at least, a combination of two or more degeneration diseases, as has been suggested by Murphy (*29, p. 69*) and by Quanjer (*39, p. 127–128*). It will be considered as such in the following section of this paper. However, a symptom complex here designated as unmottled curly dwarf of Green Mountains, has remained too true to type for three years to be considered yet as a combination of diseases, although it may eventually be demonstrated to be such. It consists of pronounced dwarfing, spindliness, dark green color of the foliage early in the season, wrinkling, rugosity, slight ruffling, curling, some rolling, uprightness, brittleness, burning, somewhat premature death, and spindling, gnarled, and cracked tubers (Pl. 11, A, B, 2, 3, 5; 12, A, 1). It may be the result of leaf-rolling mosaic and the spindling-tuber disease together with the mottling of leaf-rolling mosaic masked by the spindling-tuber disease. If so, the combination known as "mottled curly dwarf" would appear to be the same with the addition of mild mosaic, an assumpton that is hard to accept in view of the fact that the tubers are not so small and cracked in "mottled curly dwarf" as in "unmottled curly dwarf."

Leaf-mutilation inoculations were made in the open field in 1920. Inoculum was secured from an unmottled curly-dwarf hill and introduced into the second and third hills of each of four 4-hill tuber units. The symptoms appeared first in 1921, and again in 1922 in the open field, and in the winter of 1921–22 in the greenhouse, always as unmottled curly dwarf. Intervarietal transmission with aphids will be described later.

Leaf-mutilation inoculations were made in the Orono greenhouse in the winter of 1921–22 in six hills from three tuber units. The two progeny of one of the inoculated hills showed the same symptoms as the 1921 field symptoms resulting from the 1920 inoculations, even to the gnarling and cracking of the seven spindling tubers. However, the percentage of infection was much lower than for rugose mosaic in a parallel series of inoculations (p. 52).

COMPARISON OF DISTINCT DISEASES

The several symptom complexes, presumably different diseases caused by distinct though somewhat similar viruses, that have been previously considered in this paper may be compared in Table VIII. There a symptom is to be considered as absent if not noted for a disease. The

characterization given is typical of Green Mountains in northeastern Maine and does not include variations due to environmental factors, degree of maturity, and current-season inoculation. It may be pointed out here that Quanjer (*39, p. 140*) suggests the possibility of viruses adapting themselves to new varieties, and that this in turn suggests the question of change of a virus in the same variety leading to new symptom complexes somewhat related to the old, as are streak and rugose mosaic current-season symptoms, mild mosaic and leaf-rolling mosaic, curly dwarf (combination of leaf-rolling mosaic and spindling tuber) and unmottled curly dwarf. Also, a varietal modification of a virus if occurring might be permanent even when the virus is returned to the variety originally affected. It may also be suggested that different viruses or causal agents may account for the differences in symptoms.

TABLE VIII.—*Symptoms and transmission of degeneration diseases of potatoes in the Green Mountain variety*

Name of disease.	Size.	Stem texture.	Color.	Leaf texture.	Leaf form.
Mild mosaic	Slight dwarfing		Distinct mottling.	Tenderness	Wrinkling, ruffling.
Leaf-rolling mosaic.	do		Indistinct mottling.	do	Wrinkling, ruffling, rolling.
Rugose mosaic	Distinct dwarfing.	Brittleness	Mottling and slight chlorosis.	Slight stiffness	Wrinkling, rugosity.
Streak	Severe dwarfing after tuber perpetuation.	do		Tenderness after tuber perpetuation.	Wrinkling, curling, and rugosity after tuber perpetuation.
Leaf roll	Distinct dwarfing.	Rigidity	Chlorosis	Stiff and leathery.	Rolling.
Spindling-tuber disease.	Spindling		Dark green early in season.	Slight stiffness	Slight rugosity early in season.
Unmottled curly dwarf.	Severe dwarfing.	Brittleness	do	do	Wrinkling, ruffling, rolling. curling, rugosity.

Name of disease.	Necrosis.	Tuber symptoms.	Transmission demonstrated.[1]	Incubation period.
Mild mosaic			Grafts, aphids, and leaf mutilation.	Usually over 25 days.
Leaf-rolling mosaic			Aphids	Nearly like mild mosaic.
Rugose mosaic	Varying; more streaking from current-season inoculations.		Aphids and leaf mutilation.	About 14 days.
Streak	Streaking, spotting, leaf dropping, premature death.	Browning near eyes.	Leaf mutilation	About 12 days.
Leaf roll	Burning	Streaking (net necrosis).	Grafts and aphids; leaf mutilation unsuccessful.	Usually over 25 days.
Spindling-tuber disease.		Spindling	Grafts, aphids, and leaf mutilation.	Do.
Unmottled curly dwarf.	Burning, stem streaking.	Spindling, cracked.	Aphids and leaf mutilation.	Do.

[1] Including intervarietal transmission into Green Mountains, as described in this paper.

COMBINATIONS OF DISEASES

When a certain symptom complex contains the symptoms of two or more of the several distinct diseases described in the preceding pages, and is associated with them, diagnostic evidence is therefore present, indicating that the former is a combination of the latter. Analytical

evidence of such combinations has been obtained, consisting of the results of inoculations in which one or more of the combined diseases are transmitted separately to new hosts of the same variety. Such evidence easily discloses a more infectious disease in the combination but a less infectious disease will seldom be separated analytically from others not more infectious. Synthetic evidence, obtained by inoculating in such a way that new diseases are introduced into diseased plants or so that diseases are combined in healthy plants, is difficult to secure except where transmitting insects are controlled, especially when diseases are involved with a long incubation period and a consequent infrequency of current-season symptoms.

There are many theoretically possible combinations of the several diseases that have been discussed. The number of actual combinations observed by the writers has been reduced from the theoretical number by several factors. In northeastern Maine, leaf roll is rare in native stocks and often decreases or disappears in infected stocks. Many combinations quickly eliminate themselves by their excessive reduction of the yield and by causing premature death and thus reducing the chances for insect transmission. Varietal susceptibility also seems to be of influence. Furthermore, without synthetic evidence it may be assumed that the results of some combinations may be a masking of some of the symptoms of the separate diseases entering the combination. It is possible for the writers at present to show only the general significance of the principle that combinations of degeneration diseases may exist.

The writers, even with the limitations just previously set forth, have seen symptom complexes that evidently were combinations of diseases. Double combinations are more easily diagnosed than triple combinations. Several combinations are given in Table IX as having been observed by the writers. Combinations are given by Murphy (*29, p. 71*), Quanjer (*39, p. 128*), and Gilbert (*17*).

TABLE IX.—*Combinations of degeneration diseases thought to have been observed in Green Mountain potatoes*

Name of combination as formerly described.	Combined diseases.						
	Mild mosaic.	Leaf-rolling mosaic.	Rugose mosaic.	Streak.	Leaf roll.	Spindling tuber.	Unmottled curly dwarf.
	X			[a] X			
	X			[a] X		X	
	X				X		
Medium mosaic. (In part.)	X					X	
Mosaic dwarf; bad mosaic. (In part.)		X	X			X	
		X			X		
Mottled curly dwarf		X				X	
Mosaic dwarf; bad mosaic. (In part.)			X	X			
Medium plus mosaic. (In part.)			X			X	
				[a] X		X	
					X	X	

[a] Probably first-season symptoms of rugose mosaic.

As reported previously (*41, p. 54–55*), in 1919 aphids were transferred from a plant with both mild mosaic and leaf roll to three healthy hills in an insect cage, and a higher percentage of the progeny were leaf roll (86 per cent) than were mosaic (57 per cent). As is shown in Table II, aphids from hills both mild mosaic and spindling-tuber, with contact with those hills, resulted in mosaic infection of two hills, spindling tuber infection of two hills, and in no infection of a fifth hill. As described on page 51, aphids from a "mottled curly-dwarf" (or spindling tuber + leaf-rolling mosaic) hill infected a hill in an insect cage partly with curly dwarf and partly with the spindling-tuber disease alone (Pl. 3, B, 1, 2).

CONCLUSIONS REGARDING TRANSMISSION AND DIAGNOSIS IN THE GREEN MOUNTAIN VARIETY

Within one variety in a given time and place, it has been possible to distinguish diagnostically several degeneration diseases of potatoes, occurring both singly and in combinations. These several degeneration diseases can be studied to advantage by means of inoculations with grafts, aphids, and the leaf-mutilation method. In this way further distinctions can be made between the more similar diseases such as the types of mosaic, and combinations of diseases can be divided. The same methods have been used in Minnesota in this variety in "mosaic-dwarf" inoculation by grafting, leaf mutilation, root contact, and exposure to insect dispersal from diseased plants, with similar results, positive except with root contact (*21, p. 13–22*).

TRANSMISSION AND DIAGNOSIS WITHIN VARIETIES OTHER THAN THE GREEN MOUNTAIN

It is thought that the several diseases described in the preceding pages in Green Mountains are present also in other varieties. Assuming that there may be varietal modification of symptoms, as will be shown later in this paper to be possible, intervarietal inoculation is necessary for conclusive proof that a symptom complex in one variety is caused by the same virus as a symptom complex in another variety. However, it is thought that leafroll and the spindling-tuber disease can often be correctly diagnosed outside of Green Mountains without intervarietal inoculations.

In 1920, as is described partly in a previous paper (*41, p. 53*), leaf-roll inoculations were made on Irish Cobblers inside of insect cages by means of grafting, leaf mutilation, aphids, and contact. The progeny were observed in 1921, with the data given in Table X. It is clear that grafting and aphids were the only effective means employed, and that aphids were not as effective as with mosaic in the same season (Table I). Corresponding leaf-mutilation inoculations in the open field in the two hills of each of 12 four-hill tuber units, gave negative results in both generations. Kasai (*20, p. 66*) describes reduction of leaf-roll contamination by the use of field insect cages in Japan.

Leaf-mutilation inoculations were made in the Orono greenhouse during the winter of 1921–22 in Irish Cobblers, with rugose mosaic, with leaf roll, and with a combination of the two. The four hills inoculated with rugose mosaic showed leaf dropping in from 25 to 50 days. Their 12 progeny were mosaic, with leaf dropping. The four controls

in the same two tuber units were healthy, as were their 4 progeny grown in the greenhouse. Two hills had the combination of leaf roll and rugose mosaic. One of these lived longer than the three hills with rugose mosaic alone. The other was used as a source of inoculum for leaf-mutilation inoculation of two hills which showed leaf dropping in 32 and 50 days, respectively, but whose 8 progeny were healthy. Inoculation with leaf roll alone in four hills of two tuber units gave negative results which persisted in the 14 progeny. The designation of rugose mosaic here in Irish Cobblers is based on the results of parallel intervarietal inoculations to Green Mountains.

TABLE X.—*Leaf-roll inoculations of caged Irish Cobblers in 1920*

Inoculation.			Progeny, 1921.	
Series.	Method.	Hills.	Tuber units.	Leaf roll.
				Per cent.
1	Grafting stalks	6	29	100
2	None, in cages of Series 1	3	9	0
3	Leaf mutilation	4	24	0
4	Aphids and full contact	6	39	18
5	Aphids	9	51	22
6	Full contact	6	23	0
7	None, controls to Series 3, 4, and 6	9	22	0

Inasmuch as hill selections in 1919 gave rise to leaf-roll progeny in 1920 (when made next to leaf-roll hills) with a tendency to show more leaf roll and net necrosis with greater tuber weight (*41, p. 71*), similar hill selections were made in 1920. In Green Mountains, only 7 per cent of 183 progeny were leaf roll and 2 per cent net necrosis. In New White Hebrons, 28 per cent of the progeny in 1921 were leaf roll and 22 per cent were net necrosis. The relation to tuber weight is shown in Table XI. There was again an increase of each disease with an increase in tuber weight.

TABLE XI.—*Correlation in New White Hebrons in 1921, between tuber weight, net, necrosis, and leaf-roll infection, at the time of planting* [1]

Tuber weight.	Number of tubers.	Net necrosis in tubers.	Leaf roll in tubers.
Ounces.		*Per cent.*	*Per cent.*
1	8	0	0
1 to 2	19	5	11
1 to 3	29	10	17
1 to 4	36	14	22
1 to 5	42	14	21
1 to 6	47	15	21
1 to 7	52	19	25
1 to 8	54	22	28

[1] Leaf-roll infection in tubers was ascertained from the appearance of their plant progeny in the early part of the season.

INTERVARIETAL TRANSMISSION AND VARIETAL MODIFICATION OF SYMPTOMS

Observations by the writers, made largely of plants grown or furnished by the Office of Horticultural and Pomological Investigations, United States Department of Agriculture, have disclosed a great variation in apparently diseased plants in the many commercial and seedling varieties available. Many of the observations were made on an unsprayed plot, maintained for the study of resistance to late blight, *Phytophthora infestans* deBy., where mottling was not masked as much as in frequently sprayed fields. Here, both in 1920 and in 1921, there were such differences between the lots and between the hills of a given lot, as well as between the symptoms observed on one date and another in the same hills, that it seemed probable that there existed varietal susceptibility and varietal modification of symptoms. The question of the existence of such modification has been considered in experiments involved with natural uncontrolled field infection, with inoculations in the field, and with inoculations in the greenhouse.

GENERAL OBSERVATIONS IN THE FIELD

In 1919, small lots of different varieties were grown in rows alternating with rows of mild mosaic Green Mountains. As the result of infection in 1918, all the 6 Triumph lots and 12 of the 14 Green Mountain lots thus planted were mosaic in over 19 per cent of the hills and were discarded. The other 2 Green Mountain lots and 1 lot each of three other varieties in the same group (*48*)—namely, Carman No. 1, Gold Coin, and Norcross—contained no mosaic in 1919, but were sufficiently infected, presumably by insects from the alternating mosaic rows, to have 48 per cent in the Gold Coin lot in 1920, and from 70 to 87 per cent in the other lots. Twenty-one lots representing the Cobbler, Early Michigan, Rose, Early Ohio, Burbank, and Rural groups were grown in the same plot in similar alternation with mosaic Green Mountain rows. Ten of these, consisting of some of those from the Rural group, were discarded in 1920 for lack of room, when they showed no mosaic. The rest were grown between Bliss Triumph mosaic rows in 1920, and by 1921, after two years of alternate-row proximity to mosaic plants, showed from none to 12 per cent mosaic. Either the Green Mountain and Triumph groups were much more susceptible to mosaic, or they displayed different symptoms from the other groups mentioned.

INTERVARIETAL INOCULATIONS IN THE FIELD

Results previously reported consist of the transmission of mosaic (probably the rugose type) by leaf mutilation with current-season symptoms (1919) from Bliss Triumph to Green Mountain, from Irish Cobbler to Green Mountain and to Bliss Triumph, and from Green Mountain to Bliss Triumph and to Irish Cobbler (*40, p. 324–26*). The progeny of the inoculated hills were all "mosaic or mosaic dwarf" in 1920—that is, infected with mild mosaic or rugose mosaic and combinations. As indicated elsewhere, the presence of disease in the second season, following inoculation in the open field with insects uncontrolled, often is not significant because of the controls having also contracted the disease from the neighboring inoculated hills. That was the case here, showing that sometimes observations on the first generation in an experiment, including the controls, are more valuable than those on the second generation.

INOCULATIONS PERFORMED IN 1920

In 1920, leaf-mutilation inoculations were performed without any effects appearing before digging time, but some effects from the inoculations were apparent in 1921 in the second generation. The tubers were split in two and planted as two-hill tuber units. In 69 of the 90 series the juice was obtained from mosaic Green Mountains while the inoculated plants were mostly of different varieties in the Rural, Cobbler, and other groups, a few being Green Mountain controls. Progeny of these inoculated plants were diseased, with mosaic or curly dwarf, in 24 of 428 tuber units, or in about 6 per cent, and progeny of the controls in 7 of 509, or in about 1 per cent. This difference is not very significant, especially since the diseased units were not grouped in correlation with any condition of inoculation. In 21 of the 90 inoculation series the inoculated plants were Green Mountains of a certain strain while the juice or inoculum was obtained from other varieties except for a few Green Mountain controls. Five of these undoubtedly were successful in producing infection, and all are considered in detail in Table XII. Here it must be remembered that the terminology was more general regarding diseases than at present. The controls were hills in the same tuber units as the inoculated hills.

It is noteworthy that the stocks which served as the sources of inoculum in Series 5, 14, and 17 apparently recovered from mosaic in 1921. This apparent recovery was noted for other stocks, both by the writers in strains all mild mosaic for several years previously and by seed-certification officials over several States, and is to be attributed to seasonal modification or masking of symptoms. It will be discussed more fully later. It is probable that mild mosaic was not apparent even when the virus was present.

Inoculations in Series 5 and 8 were successful in inducing "mosaic," (probably not of the mild type) with symptoms in the progeny of slight dwarfing, slight wrinkling, slight burning, and mottling or chlorosis. Inoculation Series 2 and 16 would be considered successful also if the controls were healthy. Possibly the controls were infected by insects from the adjacent inoculated plants. Inoculation Series 3 and 4 were partly successful, being performed under control (insect-cage) conditions as a duplicate of Series 2. Inoculations in Series 7, 19, and 66 were successful in inducing "curly dwarf," with symptoms consisting of marked dwarfing, ruffling, curling, brittleness, stem streaking, and burning (Pl. 11, A, B, 2, 3, 5; 12, A, B). Disease appearing in 1921 as a mosaic was thus induced in Green Mountains by inoculation with juice from mosaic and curly-dwarf plants of the Rural type, and curly dwarf was induced with juice from dwarfed and wrinkled Netted Gems, curly-dwarf Green Mountains, and curly-dwarf Irish Cobblers. The third generation of representative parts of Series 8 and 19 were grown in 1922, a season unusually favorable for the detection of mosaic mottling. Series 8 contained leaf-rolling mosaic, rugose mosaic, spindling-tuber disease, and various combinations including mottled curly dwarf in the majority of the tuber units, with the controls mostly healthy. Series 19 consisted almost entirely of unmottled curly-dwarf plants (p. 60), with the controls mostly healthy. It is to be concluded that the open-field leaf-mutilation inoculations in 1920 at least transmitted rugose mosaic, a combination of leaf-rolling mosaic and spindling-tuber disease, and unmottled curly-dwarf.

TABLE XII.—*Intervarietal leaf-mutilation inoculations of Green Mountains performed in the open field in 1920*

Inoculation series.[1]	Diseased plants, source of inoculum.				Inoculated plants.				Controls.			
	Variety.	Parents.		Progeny, 1921.	1920	Progeny, 1921.			1920	Progeny, 1921.		
		1919	1920		Hills.	Tuber units.	Mosaic.	Curly dwarf.	Hills.	Tuber units.	Mosaic.	Curly dwarf.
							Per cent.	*Per cent.*			*Per cent.*	*Per cent.*
1	Russet Rural	Healthy	Healthy	Healthy	10	49	0	0	10	47	0	0
2	do	Curly dwarf	Curly dwarf	Curly dwarf	8	38	29	0	8	39	36	0
3-4	do	do	do	do	[2] 4	22	5	0	[2] 4	(?)	0	0
5	do	Mosaic	Mosaic	Apparently healthy.	10	48	67	6	9	43	7	12
6	Green Mountain		do		8	48	4	2	8	50	0	0
7	Netted Gem	Healthy	Dwarfing and wrinkling.		8	37	0	84	8	38	5	0
8	Non Blight[3]	Curly dwarf	Curly dwarf	Curly dwarf	6	29	69	0	6	25	0	0
9	Uncle Sam[3]	do	do	do	10	48	6	6	10	50	8	6
10	Rural New Yorker No. 2.	do	do	do	10	43	0	2	10	46	4	4
11	Carman No. 3[3]	do	do	do	10	47	0	0	10	41	0	0
12	Prince Henry[3]	do	do		10	47	0	4	10	47	0	0
13	Radish, control		Healthy		10	39	2	2	9	43	4	0
14	Carman No. 3[3]	Mosaic	Mosaic	Apparently healthy.	9	43	0	0	10	50	8	8
15	Green Mountain	Healthy	do		6	30	0	0	6	26	4	0
16	do	do	do		6	22	23	0	6	28	25	0
17	Prince Henry[3]	Mosaic	do	Apparently healthy.	10	41	0	0	10	42	2	0
18	Dearborn[3]	Curly dwarf	Curly dwarf	Mosaic dwarf	8	37	0	0	6	26	0	11
19	Green Mountain	Dwarfing, streaking.	do		8	32	0	63	6	29	0	0
20	do	Healthy	Mosaic		4	15	0	0	4	17	0	0
66	Irish Cobbler	do	Curly dwarf	Curly dwarf	10	44	0	66	10	50	0	0

[1] Given numerical order corresponding with chronological order of performance.
[2] Grown under insect cages.
[3] Of the Rural type when observed as healthy hills in the same lots in 1919.

TABLE XIII.—*Intervarietal inoculations of Green Mountains performed with aphids dispersing from diseased to healthy plants in insect cages in 1920*

Cage No.	Diseased plants, source of inoculum.				Inoculated plants.			Controls.	
	Variety.	Parent plants, 1919.	1920	1921	Parent plants, 1919.	1920	1921	1920	1921
1	Non Blight [1]	Curly dwarf	Curly dwarf	Curly dwarf	None	None	Mosaic in 40 per cent, wrinkling in 80 per cent.	Healthy	Healthy.
2	Sensation [1]	do	do	do	do	do	do	do	Do.
3	Uncle Sam [1]	do	do	do	do	do	Wrinkling in 50 per cent.	do	Do.
4	Rural New Yorker No. 2.	do	do	do	do	Chlorosis	Wrinkling in 83 per cent.	do	Do.
5	Carman No. 3 [1]	do	do	do	do	do	Mosaic in 33 per cent, wrinkling in all.	do	Do.
6	Dearborn [1]	do	do	do	do	None	Healthy	do	Do.
7	Seedling		Dwarfing, wrinkling, and rugosity.	Dwarfing, wrinkling, and rugosity.	do	do	do	do	Do.
8	Green Mountain	None	Curly dwarf	Dwarfing, chlorosis, brittleness, and burning.	do	do	do	do	Do.
9	Seedling		Wrinkling, rugosity, and slight streaking.	do	do	do	do	do	Do.
10–14	Green Mountain	Mosaic	Mosaic	Dwarfing, wrinkling, and burning.	do	do	Mosaic in 83 per cent.	do	Do.

[1] Of the Rural type when observed as healthy hills in the same lot in 1919.

During the same season, in each of nine field insect cages (No. 1 to 9 of Table XIII), a Green Mountain plant was grown between two plants that were of another variety and that apparently were diseased. Cages 10 to 14 contained comparison inoculations from mild mosaic Green Mountains. In June, potato aphids were transferred from rose bushes (*36*) to healthy potato plants (in entirely healthy tuber units and caged) and in July from these to the cages used in this experiment. The aphids were allowed to disperse freely within the cages. Sister hills in the same tuber units were grown under separate insect cages as controls, and were not inoculated. The results are given in Table XIII. It is of interest that inoculations 1, 2 and 5, were successful in inducing mosaic in the progeny, although the source of the inoculum was curly-dwarf plants. Furthermore, inoculations 1, 3, 4, and 6 in the insect cages (Table XIII) were, respectively, duplicates in a measure of the open-field inoculation Series 8, 9, 10, and 18 (Table XII). That is, the source of the inoculum in each pair of corresponding inoculations was the same stock of potatoes. The first of these four sources yielded inoculum that was ineffective with either method of inoculation, but not the others. This indicates that it was the nature of the inoculum rather than the method of inoculation that determined the success secured in the attempts at infection. The recording of more mosaic in the progeny of the caged hills than of the open-field hills is correlated with more frequent examination, made desirable by the limited number of caged hills and the expense of cage experimentation and possibly with more favorable conditions for infection the preceding season. Even so, the symptoms were not very distinct or extensively distributed over the plants.

Further open-field leaf-mutilation inoculations were made in 1920 with the inoculum taken from leaf-roll Irish Cobblers to Green Mountains in two hills of each of six four-hill tuber units, with negative results in both generations.

INOCULATIONS PERFORMED IN 1921

In 1921 the leaf-mutilation method was used again with inoculum obtained from several sources. The inoculum in each series was applied to two or more varieties, and with the exceptions of Series 1 and 7 was unrenewed—that is, the juice was expressed from a certain group of shoots at one time. The inoculated plants were in rows of 11 four-hill tuber units. One hill in each unit was inoculated with juice from mosaic Bliss Triumph plants and another with juice not alike, in regard to source, for two rows of any one variety. The date of inoculation was from July 4 to July 9, except for Series 6 and 8, for which it was July 19. Additional data are given in Table XIV. The current-season symptoms make it evident that the inoculum used in Series 1 to 5 was very infective and injurious, while that used in Series 6, 7, and 10 was apparently without effect. In general, the effects of inoculation in Series 1, 2, and 3 were similar, being those of rugose mosaic in the upper part of the plant, with the mottling fading, as often occurs, into diffused chlorosis with greater degree of maturity (Pl. 12, C). The effects of inoculation in Series 5 were different, being those of streak in the upper part of the plant (Pl. 5, A, C). Series 4 gave effects of both rugose mosaic and streak.

The second-season symptoms in 1922 are also noted in Table XIV. No distinction was attempted between the progeny of inoculated and uninoculated hills as to the spindling-tuber disease in any of the three varie-

ties or as to mild mosaic in Green Mountains, for reasons given in the footnotes. It was obvious that rugose mosaic had been transmitted to all three varieties from Rurals, and from two seedling varieties to two inoculated varieties (Series 1, 2, and 3), and that streak and the streak combination with rugose mosaic, after transmission and tuber perpetuation, were much worse in their effects than rugose mosaic alone. Leaf roll was not transmitted. Leaf-rolling mosaic from the Rurals (Series 1) was combined with spindling-tuber disease, giving mottled curly dwarf.

The conclusions from this experiment are that leaf-roll was not transmissible under the prevailing conditions; that mild mosaic and spindling-tuber disease were present and uncontrolled; that leaf-rolling mosaic was transmitted from curly-dwarf Rurals in combination with rugose mosaic and possibly with spindling-tuber disease, being combined with the latter in Green Mountains to form mottled curly-dwarf; that rugose mosaic was transmitted from four dissimilar symptom complexes in three varieties; and that streak, both alone and with rugose mosaic, was transmitted and was perpetuated by the tubers, with effects in the second generation similar for the single and combined state and at the same time unlike the effects in the first generation.

Several other interesting facts may be noteworthy. In a number of tuber units (Table XIV, rows 1–B, 1–D, and 2–J), mosaic was perpetuated by the seed tuber and was present when a more severely injurious disease was introduced to some of the hills. The inoculated hills (not described in Table XIV) became different from the others in these units after the incubation period, and the original mosaic symptoms were somewhat obscured. In Series 1 and 3 the Irish Cobblers showed fine or small spotting, but not the Green Mountains. Such spotting is common on Irish Cobblers which are found diseased as the result of natural field infection, presumably following rugose-mosaic transmission by insects. In Series 1 the Rurals showed streaking which was not in the other varieties, but they were inoculated with juice taken two days later from different hills in the same lot, so a different virus may have been present. Variation in completeness of symptom complexes in the same row following the introduction of the same inoculum was observed, as described in Table XV. Such variation may be due only to differences in the size of the plants at the time of inoculation, and therefore be due to the leaf-surface conditions of inoculation rather than to differences in the inherent nature of the plants.

TABLE XIV.—*Leaf-mutilation inoculations of Green Mountains and other varieties performed in the open field in 1921*

Inoculation series.	Diseased plants, source of inoculum.			Inoculated plants, 1921.				Uninoculated controls, 1921.		Remarks, 1921.	Progeny of inoculated plants, 1922.	Progeny of uninoculated controls, 1922.
	Variety.	Parent plants, 1920.	1921	Variety.	Row.	Hills.	Symptoms.	Hills.	Symptoms.			
1	Carmen No. 3.[a]	Curly dwarf.	Curly dwarf.	Green Mountain.	1-B.	7	Mosaic in upper leaves of some stalks by July 23, in 6 hills, chlorosis becoming diffused by Aug. 23, with brittleness and burning. One hill healthy at first but like others by Aug. 23, except not brittle.	14	None	Four tuber units mosaic.	Rugose mosaic, leaf-rolling mosaic, and spindling-tuber disease in different combinations.	No rugose or leaf-rolling mosaic.[b]
				Irish Cobbler	2-B.	11	Mosaic in upper leaves of some stalks by July 23, in 3 hills, with fine spotting by Aug. 1. Dwarfing, brittleness, chlorosis, and burning in 9 hills by Aug. 23, with wrinkling in 6 hills and fine spotting in 5 hills. One hill mottled and one healthy on Aug. 23.	22	do		Rugose mosaic[c].	No rugose mosaic.[c]
				Rural New Yorker.	3-B.	11	Mosaic and streaking in upper leaves in 7 hills by Aug. 1. On Aug. 23, 6 hills with dwarfing, brittleness, chlorosis, and burning, including 2 hills with mottling, streaking, and wrinkling also; 2 hills with mottling, streaking, and wrinkling above; 3 hills healthy.	22	do		do	Healthy.[d]
2	Seedling 41046.		Slight dwarfing, rugosity.	Green Mountain.	1-O.	10	Mosaic in upper leaves by July 23, with leaf dropping by Aug. 1, in all hills. On Aug. 23, mostly like hills of row 1-B (Table XV).	20	do		do	No rugose mosaic.[b]
				Irish Cobbler	2-O.	11	As for row 1-O except more like row 1-B finally.	22	do		do.[c]	Do.[c]

[a] Inoculum taken two days later for the Rurals, and from different hills than for the other two varieties.
[b] Much mild mosaic and spindling-tuber disease in this variety in these progeny.
[c] All spindling tuber in this variety in these progeny.
[d] No attempt to diagnose spindling-tuber disease in this variety in these progeny.

TABLE XIV.—*Leaf-mutilation inoculations of Green Mountains and other varieties performed in the open field in 1921*—Continued

Inoculation series.	Diseased plants, source of inoculum. Variety.	Parent plants, 1920.	1921	Inoculated plants, 1921. Variety.	Row.	Hills.	Symptoms.	Uninoculated controls, 1921. Hills.	Symptoms.	Remarks, 1921.	Progeny of inoculated plants, 1922.	Progeny of uninoculated controls, 1922.
3	Seedling 41046.	....	Slight dwarfing, burning.	Green Mountain.	1–H.	11	As for row 1–O except leaf dropping not present until after Aug. 1 (Table XV).	22	None........	....	Rugose mosaic..	No rugose mosaic.[a]
				Irish Cobbler	2–H.	11	Nine hills as for row 2–B. Two hills healthy July 23, but on Aug. 23, like others with fine spotting still showing.	22	.....do........	....	.....do.[b]........	Do.[b]
4	Seedling 41186.	....	Mosaic dwarf.	Green Mountain.	1–D.	9	Eight hills mosaic in upper leaves by July 23, with spotting in 6 hills, and with leaf dropping, spotting, and streaking by Aug. 1; dead by Aug. 23. One hill healthy at first but with brittleness, streaking, and burning by Aug. 23.	17	Three hills mosaic.	Two tuber units mosaic.	Severe dwarfing and early death; second season streak symptoms.	No dwarfing.[a]
				Irish Cobbler	2–D.	11	All hills mosaic in upper leaves by July 23, with spotting or streaking or both. By Aug. 23, either dead or like row 2–B.	22	None........	....	.....do.[b].......	Do.[b]
5	.....do......	....	Streak.......	Green Mountain.	1–J..	10	Spotting and streaking in 5 hills by July 23, and in 8 hills by Aug. 1, with death of upper parts of some shoots. Six hills dead by Aug. 23, and 3 hills with brittleness, burning, spotting, and streaking.	19	.....do........	....	.....do..........	Do.[a]
				Irish Cobbler	2–J..	10	As for row 1–J except 9 hills affected by July 23 and all hills by Aug. 1, and 8 hills dead on Aug. 23.	22	.....do........	One tuber unit mosaic.	.....do.[b].......	Do.[b]

				Green Mountain.	1-Q.	8	None.	24	....do.	Six tuber units mosaic.	Mild mosaic [a] ...	Mild mosaic.
				Irish Cobbler	2-Q.	11	....do.	33	One hill streak in part.	....	No mosaic [b].....	No mosaic.[b]
6	Green Mountain.	Part mosaic.	Mosaic	Rural New Yorker.	3-J..	11	....do.	33	None.	....	Healthy [c].......	Healthy.[c]
				Inoculated controls.[d]	Inoculated controls.[d]	110	....do.	[e] 0	....	....	No rugose mosaic.[a b]	No rugose mosaic.[a b]
7	Bliss Triumph.	Mosaic......	Mosaic.......	Bliss Triumph.	3-N.	9	....do.	27	....do.	....	Healthy [c].......	Healthy.[c]
8	....do.[f]....	....	....do.......	Green Mountain.	Cages M-1,2.	6	Mosaic in upper leaves of 2 hills	6	....do.	....	28 per cent mild mosaic.	Do.
				....do.......	1-D.	9	Mosaic in upper leaves of 2 hills (field infection).	8	Mosaic in part in 1 hill.	....	No rugose mosaic.[a]	No rugose mosaic.[a]
9	Water controls.	....	....	Irish Cobbler	2-D.	11	None.	11	None.	....	....do.[b]........	Do.[b]
				Rural New Yorker.	3-C.	11	....do.	33	....do.	....	....do.[c]........	Do.[c]
				Green Mountain.	1-F.	11	....do.	22	....do.	....	No leaf roll [a]	No leaf roll.[a]
10	Irish Cobbler	Leaf roll.....	Leaf roll.....	Irish Cobbler	2-F.	11	....do.	22	....do.	....	....do.[b]........	Do.[b]
				Rural New Yorker.	3-N.	?	....do.	?	....do.	....	....do.[c]........	Do.[c]

[a] Much mild mosaic and spindling-tuber disease in this variety in these progeny.
[b] All spindling tuber in this variety in these progeny.
[c] No attempt to diagnose spindling-tuber disease in this variety in these progeny.
[d] Hills adjacent to those inoculated in series 1 to 5.
[e] Same as for series 1 to 5.
[f] The same unrenewed inoculum that was used without visible effects on hills in row 3-N (Bliss Triumph), 1-M (Green Mountain), and 2-M (Irish Cobbler).

Tavle XV.—*Symptoms of inoculated hills of rows 1–0 and 1–H of Table XIV, on August 23*

Row.	Tuber unit.	Dwarfing.	Chlorosis.	Mottling.	Wrinkling.	Rugosity.	Ruffling.	Curling.	Rolling.	Uprightness.	Rigidity.	Brittleness.	Spotting.	Streaking.	Burning.	Leaf dropping.	Premature death.
1–O	1	+	+	....	+	....	....	....	....	....	....	+	....	....	+	+	....
	2	+	+	....	+	....	....	....	....	....	....	+	....	....	+	+	....
	3	+	....	+	+	....	....	....	....	....	....	....	....	....	+	+	....
	4	+	....	+	+	....	....	+	....	....	....	+	....	....	+	+	....
	5	+	....	+	+	....	....	....	....	....	....	....	....	....	+	+	....
	6	+	....	+	+	....	....	....	....	....	....	+	....	....	+	+	....
	7	+	+	....	+	....	....	....	....	....	....	....	....	....	+	+	....
	8	+	+	....	+	....	....	....	....	....	....	+	....	....	+	+	....
	9	+	+	+	+	....	....	....	....	....	....	....	....	....	+	+	....
	10	+	....	+	+	....	+	....	....	....	....	+	....	....	+	+	....
1–H	1	+	....	+	+	....	....	....	....	....	....	+	....	....	+	+	....
	2	+	+	....	+	....	....	....	....	....	....	+	....	....	+	+	....
	3	+	+	....	+	....	....	....	....	....	....	+	....	....	+	+	....
	4	+	....	+	+	....	....	....	....	....	....	+	....	....	+	+	....
	5	+	+	....	+	....	....	+	....	....	....	....	....	....	+	+	....
	6	+	+	+	+	....	....	....	....	....	....	+	....	....	+	+	....
	7	+	+	+	+	....	+	....	....	....	....	+	....	....	+	+	....
	8	+	....	+	+	....	+	....	....	....	....	....	....	....	+	+	....
	9	+	....	+	+	....	....	+	....	....	....	....	....	....	+	+	....
	10	+	....	+	+	....	+	....	....	....	....	....	....	....	+	+	...
	11	+	....	+	+	....	....	+	....	....	....	....	....	....	+	+	....

In connection with the preceding experiment, duplicates of many of the leaf-mutilation inoculations were made with capillary glass tubes. From three to five of these tubes were filled with the juice and inserted into each shoot in the leaf axils (Pl. 13, B). This method was effective only in the rows corresponding to 1–O, 2–O, and 1–J (Table XIV), and there only in a few hills, with symptoms appearing later than in the case of leaf mutilation. Apparently, transmission is too difficult with this method to be dependable, at least in comparison with the leaf-mutilation method.

Corresponding inoculations of caged Green Mountains and Irish Cobblers with potato aphids, with contact of roots and foliage, were made in 1921. Six tubers of each variety were quartered. The quarters from each tuber were grown, respectively, in cages of four series—with no inoculation, with inoculation from mosaic Green Mountain hills, with inoculation from curly dwarf hills, and with inoculation from progeny of streak hills. In the first series there were three healthy plants in a cage, in the second there were two healthy and one diseased in a cage, and in the two others one healthy and two diseased. The aphids were transferred to the diseased plants and in turn reached the healthy plants in the same cages. The latter were planted somewhat later than the former so that they were still small when aphids were numerous enough to disperse and to be transferred artificially. Some of the diseased plants in the streak series were removed on account of the appearance of mottling and the cages used for other experiments. This experiment is described further in Table XVI.

TABLE XVI.—*Inoculation of Green Mountains and Irish Cobblers performed with aphids dispersing from diseased to healthy plants in insect cages in 1921*

Inoculation No.	Diseased plants, source of inoculum.				Inoculated plants.			
	Variety.	Parent plants, 1920.	1921	1922.[a]	Variety.	Parent plants, 1920.[b]	1921	1922.[a]
1	Russet Rural	Curly dwarf	Curly dwarf	Curly dwarf	Green Mountain	Healthy	Mosaic in upper leaves.	5/5 leaf-rolling mosaic.
			do	do	Irish Cobbler	do	Healthy	1/2 dwarfed.
2	Uncle Sam [c]	do	do	do	Green Mountain	do	Mosaic in upper leaves.	5/5 leaf-rolling mosaie (Pl. 2, C, 1).
			do	do	Irish Cobbler	do	Healthy	2/3 wrinkled.
3	Rural New Yorker No. 2.	do	do	do	Green Mountain	do	do	1/11 leaf-rolling mosaic.
			do	do	Irish Cobbler	do	do	2/2 dwarfed.
4	Irish Cobbler	do	do	Dwarfed	Green Mountain	do	do	3/3 unmottled curly dwarf (Pl. 10, A, 2).
5[d]	Green Mountain	Mosaic and spindling tuber [b]	Mosaic and spindling tuber.	Mild mosaic and spindling tuber.	do	do	do	6/19 mild mosaic; 5/19 spindling tuber.
6	do	do	do	do	Irish Cobbler	do	do	Healthy.
7	Seedling 39496	Curly dwarf	Dwarfing, leaf dropping, and premature death.	Dwarfing	Green Mountain	do	Mosaic in upper leaves of part, and later streaking also.	2/6 rugose mosaic (Pl. 4, A, 1).
			Dwarfing, mottling, brittleness, leaf dropping, and premature death.	do	Irish Cobbler	do	Streaking	1/2 rugose mosaic.
8	Green Mountain	do	Curly dwarf	Leaf-rolling mosaic and spindling tuber (curly dwarf combination).	Green Mountain	do	Healthy	1/3 spindling tuber; 2/3 spindling tuber and leaf-rolling mosaic (curly dwarf combination (see Pl. 3, A, B).
			do	do	Irish Cobbler	do	do	2/2 wrinkled.
9	do	do	do	Dwarfed	do	do	do	6/6 wrinkled.
10	Seedling 39374	Streaking	Mosaic	Rugose mosaic	Green Mountain	do	do	6/6 healthy.
			do	do	Irish Cobbler	do	do	Healthy.
11	Seedling 39937	do	Dwarfing, rugosity, brittleness, burning.	Curly dwarf and rugose mosaic.	Green Mountain	do	Mosaic in upper leaves, becoming mosaic-dwarf.	4/4 leaf rolling and rugose mosaic.
			do	do	Irish Cobbler	do	do	4/4 rugose mosaic.
12	Seedling 40203	do	Dwarfing, ehlorosis, rugosity, burning.	Dwarfed, rugose	Green Mountain	do	Mosaic in upper leaves.	2/2 leaf-rolling and rugose mosaic.
			Chlorosis	do	Irish Cobbler	do	Healthy	5/5 dwarfed.
13	Charles Downing Irish Cobbler		Streaking Spindling tuber	Rugose mosaic Spindling tuber	Green Mountain	do	do	4/4 spindling tuber.
14	Green Mountain	Streaking	Mosaic	5/5 mild mosaic; 3/5 spindling tuber	do	do	Mosaic in upper leaves.	4/4 mild mosaic and spindling tuber (see Pl. 8, B).
					Irish Cobbler	Spindling tuber.	Spindling tuber	2/2 wrinkled and spindling tuber.

[a] Mostly 2-hill tube units. [b] Caged and not inoculated. [c] Of the Rural type when observed as healthy hills in the same lot in 1919. [d] Also described in Table II.

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Current-season symptoms appeared in some of the cages in which the disease present was curly dwarf (inoculation Series 1 and 2, Table XVI) and in one of the cages in which the disease was mosaic (No. 14), resulting in mosaic in the upper leaves. Mosaic also resulted from No. 12 in which the symptoms were dwarfing, chlorosis, rugosity, and burning, from No. 11 in which there were dwarfing, brittleness, rugosity, and burning, and from No. 7 in which there were dwarfing, leaf dropping, and premature death. However, in No. 11 the final effect was mosaic-dwarf, and in No. 7 it was a combination of mosaic and streaking. Inoculation was effective in the Irish Cobblers only in No. 11 and 7, the results being similar to those in the other variety except for the absence of mosaic in No. 7.

The second generation was planted in the open field and the symptoms are also given in Table XVI except that all the inoculated Irish Cobblers were spindling tuber and likewise the controls in this variety. The Green Mountain controls were all healthy. It appears that the inoculation of Green Mountains resulted in the transmission of leaf-rolling mosaic in No. 1, 2 (Pl. 2, C, 1,), and 3, from curly dwarf Rurals; of rugose mosaic in No. 7 from dwarfed seedling variety 39496 (Pl. 4, A); of spindling-tuber disease in No. 5, 8 (Pl. 3, B, 1) and 13 from spindling-tuber Green Mountains and Irish Cobblers; of mild mosaic in No. 5 from mild mosaic Green Mountains; of unmottled curly dwarf in No. 4 from Irish Cobblers (Pl. 10, A, 2,) and of various combinations of these four diseases in No. 8 (Pl. 3, B), 11, 12, and 14 (Pl. 8, B). It is noteworthy that with earlier transmission and consequent more current-season symptoms in 1921, there was more complete infection of the progeny in 1922. Infection of the Irish Cobblers, already spindling tuber, was not so apparently successful. In inoculations No. 1, 2, 3, 4, 7, and 12, the leaf-rolling mosaic, rugose mosaic, or unmottled curly dwarf symptoms induced in the Green Mountains were not like the symptoms in the source of inoculum hills or their progeny, suggesting varietal modification and masking of symptoms, especially of mosaic (Pl. 2, C; 4, A). The inoculated hills in No. 7, the parents of the diseased hills in No. 10 and 14, and the Charles Downing diseased hill in No. 13, showed streaking which was replaced in the following generations by rugose mosaic or by mild mosaic in combination with spindling-tuber disease.

INOCULATIONS PERFORMED IN 1922

In the field, during 1922, leaf-mutilation inoculations with different types of mosaic, with leaf roll, and with spindling tuber were made on plants of the Green Mountain, Irish Cobbler, Bliss Triumph, and Rural New Yorker varieties. The method of planting, in 4-hill tuber units, resembled that of previous seasons. One or two hills in a tuber unit, either the second and third hill, or the third hill alone, were treated so that two or three uninoculated controls were in each inoculated tuber unit. Single applications were made in each series with the exception of Series 58, 59, and 60, in which the second hill in each unit received two inoculations, and with the exception of mild mosaic and leaf roll, where three repeated applications were made. The positive current-season results of these inoculations are presented in Table XVII.

Inoculations with mild mosaic and leaf roll are not indicated in this table, since no current-season symptoms appeared. Infection from inoculations made under cages with mild mosaic by leaf mutilation is described in Table XVIII. Inoculations with spindling tuber were discussed in Table VII.

TABLE XVII.—*Leaf-mutilation inoculations of Green Mountain and other varieties, performed in the open field in 1922*

Inoculation series.[1]	Source of inoculum.			Inoculated plants.				Uninoculated controls.	
	Variety.	Symptoms. Parents, 1921.	Symptoms. Diseased plants, 1922.	Variety.	Row.	Hills.	Symptoms.[2]	Hills.	Symptoms.
10	Greeu Mountain	Rugose mosaic and streak.[3]	Severe dwarfing and early death.	Green Mountain	2; sec. 8	5	Rugose mosaic and streak	15	Healthy.
11				Irish Cobbler	2; sec. 7	5	do	15	No rugose mosaic or streak.
12				Bliss Triumph	1; sec. 6	5	Rugose mosaic and streak in 2 hills	15	Do.
14	do	Streak [4]		Green Mountain	2; sec. 8	5	Streak	15	No streak.
15				Irish Cobbler	2; sec. 7	5	Streak in 3 hills	15	Do.
16				Bliss Triumph	1; sec. 6	5	Streak in 4 hills	15	Do.
18	do	Rugose mosaic [5]	Leaf-rolling mosaic and spindling tuber (curly dwarf).	Green Mountain	3; sec. 8	5	Rugose mosaic and streaking	15	No streaking; 2 hills rugose mosaic.
19				Irish Cobbler	3; sec. 7	5	do	15	No rugose mosaic or streaking.
29				Bliss Triumph	2; sec. 6	5	Rugose mosaic and streaking in 3 hills.	15	Do.
58	do	do	Rugose mosaic	Green Mountain	9; sec. 8	10	Rugose mosaic	10	No rugose mosaic.
59				Irish Cobbler	9; sec. 7	10	do	10	Do.
60				Bliss Triumph	8; sec. 6	10	Rugose mosaic in 9 hills	10	Do.
79	do	Rugose mosaic [6]	Bad mosaic and spindling tuber.[7]	Green Mountain	11; sec. 8	5	Rugose mosaic in 3 hills	15	Do.
80				Irish Cobbler	11; sec. 7	5	do	15	Do.
81				Bliss Triumph	9; sec. 6	5	Rugose mosaic and streaking in 3 hills	15	Do.
83	Rural New Yorker	Dwarfing and leaf rolling.	Rugose mosaic and leaf-rolling mosaic.	Green Mountain	12; sec. 8	5	Rugose mosaic	15	Do.
84				Irish Cobbler	12; sec. 7	5	Rugose mosaic in 3 hills	15	Do.
85				Bliss Triumph	10; sec. 6	5	Rugose mosaic and streaking	15	Do.
87	Green Mountain	Rugose mosaic[8]	Rugose mosaic	Green Mountain	12; sec. 8	5	Rugose mosaic	15	Do.
88				Irish Cobbler	12; sec. 7	5	do	15	Do.
89				Bliss Triumph	10; sec. 6	5	Rugose mosaic and streaking in 4 hills	15	Do.
105	Seedling 39374		Dwarfing; no mottling.	Green Mountain	15; sec. 8	5	Rugose mosaic and streaking	15	Do.
106				Irish Cobbler	14; sec. 7	5	do	15	Do.
107				Rural New Yorker	12; sec. 5	5	Streak in 3 hills	15	No streaking.

[1] Each group of consecutive numbers represents a group of series with identical inoculum.
[2] In all inoculated plants, unless number is stated.
[3] Row 1-D of Table XIV.
[4] Row 1-J of Table XIV.
[5] Row 1-B of Table XIV.
[6] Row 1-H of Table XIV and Table XV, tuber unit 9.
[7] Thought to contain leaf-rolling mosaic as well as rugose mosaic.
[8] Row 1-H of Table XIV and Table XV, tuber unit 10.

TABLE XVII.—*Leaf-mutilation inoculations of Green Mountain and other varieties, performed in the open field in 1922*—Continued.

Inoculation series.[1]	Source of inoculum.			Inoculated plants.				Uninoculated controls.	
	Variety.	Symptoms.		Variety.	Row.	Hills.	Symptoms.[2]	Hills.	Symptoms.
		Parents, 1921.	Diseased plants, 1922.						
123	Seedling 39374	....	Rugose; leaf dropping.	Green Mountain	20; sec. 8	5	Rugose mosaic and streaking	15	No rugose mosaic and no streaking.
124				Irish Cobbler	15; sec. 7	5	do	15	Do.
127	Green Mountain	....	Bad curly dwarf and leaf dropping.	Green Mountain	21; sec. 8	5	Rugose mosaic and streaking in 3 hills	15	Do.
128				Irish Cobbler	16; sec. 7	5	Rugose mosaic and streaking	15	Do.
130	Seedling 41451	....	Streak and slight rugosity on young leaves.	Green Mountain	21; sec. 8	5	Rugose mosaic and streaking in 3 hills	15	Do.
131				Irish Cobbler	16; sec. 7	5	Rugose mosaic and streaking	15	Do.
133	Seedling	....	Streaked and no apparent mottling.	Green Mountain	22; sec. 8	5	Rugose mosaic and streaking in 2 hills	15	Do.
111	Rural New Yorker	....	Curly dwarf	do	16; sec. 8	5	Rugose mosaic and streaking	15	Do.
119	do	....	Curly dwarf; mottled.	do	18; sec. 8	5	Rugose mosaic and streaking in 4 hills.	15	Do.

[1] Each group of consecutive numbers represents a group of series with identieal inoculum.
[2] In all inoculated plants, unless number is stated.

As indicated in Table XVII, the current-season reactions of the varieties inoculated with juice from vines showing different types of mosaic, dwarf, and streak symptoms, were rugose mosaic and streak or combinations of these. Streak alone resulted from inoculations with juice from a Green Mountain lot inoculated in 1921 with juice from a streak plant in a seedling variety (Series 14, 15, and 16). Streak, therefore, has appeared without apparent combinations in three succeeding generations, including two in Green Mountains and Irish Cobblers as the result of leaf-mutilation inoculation. As indicated, the second generation plants used as source of inoculation for streak in 1922, were badly dwarfed and died early—before tuber formation. Similar phenomena were obtained with the progeny of plants inoculated in 1921, with juice from a mosaic dwarf plant adjacent to the streak plant, of the seedling variety, mentioned above (Series 10, 11, and 12). Here some of the inoculated hills produced stalks showing streak alone and rugose mosaic alone, as well as a combination of these symptoms, suggesting that mosaic-dwarf in the original seedling plant may have been a combination of rugose mosaic and streak, the severe dwarfing being due mainly to streak in the combination. These two inoculations are different from the others in that they produced streak alone in Green Mountains and Irish Cobblers while the others produced rugose mosaic in close association with any streaking seen in these varieties. They are also different because the source of inoculum was extremely dwarfed plants quite unlike the plants, with rugose mosaic or other symptom complexes, that served as sources of inoculum for the other inoculations.

It seems probable that streaking may be a prominent first-season symptom of two diseases—streak and rugose mosaic—and not always a sign of streak. This view of the uncertain value of the one symptom of streaking as a sign of the symptom complex or disease of streak is pointed out by Orton (*33, p. 100*), and is supported by the writers' comparison of first-season and second-season symptoms in a number of lots with rugose mosaic the second season. Streaking was more common as an apparent first-season symptom of rugose mosaic in Bliss Triumphs and Rurals (Table XVII, Series 81, 85, 89, and 107). Whether other diseases in several combinations used here (Series 18 to 20, 79 to 81, 83 to 85, and perhaps others) were transmitted along with rugose mosaic and merely did not show current-season symptoms, can be learned only from the second generation in 1923. The consistent emergence, from combinations, of rugose mosaic in 1922, indicates that the other diseases, whether also transmitted or not, were of a less virulent type. Modification of rugose mosaic symptoms in combination with other diseases occurred in Series 10 to 12, 18 to 20, 79 to 81, and 127 to 128. The same thing, or else varietal modification of rugose mosaic symptoms, was shown in Series 105 to 107, 123 to 124, 130 to 131, 133, 111, and 119.

In addition to those in the open field, other leaf-mutilation inoculations were made in insect cages located in the same field. As indicated in Table XVIII, repeated inoculations with mild mosaic and spindling-tuber were made in Series A–III and B–III, while but a single application was made in the remaining series, which included juice from mosaic dwarf and streak as well as from mild mosaic and spindling-tuber plants. Plants for the source of mild mosaic and spindling-tuber inoculum were grown in cages since 1920, inclusive, and for mosaic dwarf and streak since 1921. As indicated, three different varieties and one seedling were

TABLE XVIII.—*Leaf-mutilation inoculations of Green Mountain and other varieties performed in insect cages in the field in 1922*

Inoculation series.	Source of inoculum.			Inoculated plants.			Uninoculated controls.	
	Variety.	Symptoms.		Variety.	Hills.	Symptoms.	Hills.	Symptoms.
		Parents, 1921.	Diseased plants, 1922.					
A-III [1]	Green Mountain	Mild mosaic and spindling tuber.	Mild mosaic and spindling tuber.	Green Mountain	3	Mild mosaic; spindling and flat, oblong tubers in each hill.	3	No mosaic and no spindling tuber.
B-III [1]	do	do	do	Irish Cobbler	3	No apparent mottling; spindling tubers in hills 1 and 2; normal and spindling tubers in hill 3.	3	No apparent mottling; spindling tubers in hills 1 and 2; normal tubers in hill 3.
E-III	do	do	do	Green Mountain	3	Mild mosaic and spindling tuber in hill 3 (Pl. 9, B, 2); hills 1 and 2 healthy.	3	Healthy.
F-III	do	do	do	Irish Cobbler	3	No apparent mottling; normal tubers in hill 1; spindling tubers in hill 2; spindling and normal in hill 3.	3	Apparently healthy plants in hills 1 and 3; spindling tubers in hill 2.
G-III	do	do	do	Seedling 39374	3	No apparent mottling	3	Healthy.
H-III	do	do	do	Bliss Triumph	3	do	3	Do.
E-II	do	do	Mosaic dwarf	Green Mountain	3	Rugose mosaic	3	Do.
F-II	do	do	do	Irish Cobbler	3	Healthy	3	Do.
G-II	do	do	do	Seedling 39374	3	Spotting and streaking on hill 3	3	Do.
H-II	do	do	do	Bliss Triumph	3	Healthy	3	Do.
E-I	Seedling 39374	Streak	Streak	Green Mountain	3	do	3	Do.
F-I	do	do	do	Irish Cobbler	3	do	3	Do.
G-I	do	do	do	Seedling 39374	3	Streak and mottling	3	Do.
H-I	do	do	do	Bliss Triumph	3	Healthy	3	Do.

[1] 3 inoculations at weekly intervals; 1 inoculation on remaining series.

inoculated in comparative series. The average height of the plants at the time of the first inoculation was about 7 cm.

Repeated applications with mild mosaic and spindling tuber were more effective than single treatments. The Irish Cobbler apparently shows greater susceptibility to spindling tuber than to mild mosaic. A distinct varietal reaction to mosaic dwarf appears between Green Mountain and seedling 39374, being rugose mosaic or streaking and spotting, respectively, suggesting the combination for mosaic dwarf to be rugose mosaic and streak. The occurrence of spindling tuber in the controls in the Irish Cobbler variety resulted from field infection in 1921. Since in each series, hills 1, 2, and 3 in the inoculated lot are progeny from seed pieces of the same three tubers, respectively, as hills 1, 2, and 3 of the control lot, it is quite apparent that spindling-tuber infection resulted from inoculation in some units in which the controls remained healthy (Series A–III, all three hills; B–III, hill 3; E–III, hill 3 (Pl. 9, B); F–III, hill 3.)

INTERVARIETAL INOCULATIONS IN THE GREENHOUSE

Results previously reported from greenhouse experiments on intervarietal inoculations consist of the transmission of mosaic by juice transfer from Green Mountains to Bliss Triumphs (*45, p. 253–55*), and of leaf roll by aphid dispersal from both Green Mountains and Irish Cobblers to each of these varieties (*41, p. 57–58*).

In the Orono greenhouse in the winter of 1920–21, mild mosaic inoculations of Green Mountains were made with several juice-transfer methods (Table XIX, Series 1 to 10). The inoculum was always obtained from mild mosaic Bliss Triumph hills.

It will be noted in Table XIX that in Series 1 to 7 inoculation of any type was performed at four different times. The inoculated plants in this experiment were potted separately but consisted of 4-hill tuber units. The four plants grown from any one tuber were inoculated, respectively, at the four different times which were not absolute dates but corresponded to certain stages of development of the plant. The first plant to reach a height of 10 to 15 cm., or Stage I, was inoculated immediately. All four plants were observed in regard to the time when the terminal shoot exposed the first flower-bud cluster by opening and growing beyond it, and this was considered as the anthesis stage (II) since all potato buds abort in the Orono greenhouse at this season. The first plant of each tuber unit to reach the stage of anthesis, disregarding the one already inoculated at Stage I, was inoculated at that time, the next one to do so was inoculated 15 days after anthesis (at Stage III), and the last one was inoculated 30 days afterwards (at Stage IV). All were dug at Stage VIII, which was 40 days after the so-called anthesis, or II, 25 days after III, and 10 days after IV. The date of anthesis was not the same for all plants of a tuber unit, differing from 1 to 13 days, the same as for the different tuber units. In Series 8 to 10, inoculation was performed at the same stage of maturity—namely, at anthesis, but the tubers were dug at four different times for each tuber unit. The four plants were harvested, respectively, 10, 20, 30, and 40 days after anthesis, which stages are denoted, respectively, as V, VI, VII, and VIII. The later the date of anthesis the shorter interval of time was allowed between anthesis and harvesting, to make the dates of harvesting as late and as close together as possible. The tubers planted were dug in the field during the last week of July, 1920, and were planted November 15 to 19.

The plants in Series 1 to 10 reached Stage I during the last half of December and Stage II about the middle of January, and were dug up mostly in February. The progeny were planted in the field in May and were observed in August, which was cool, when they showed more distinct mottling than the all-mosaic stocks that were observed in June and July, which were warm and dry, at corresponding stages that are usually the best for mosaic diagnosis.

TABLE XIX.—*Inoculations of Green Mountains in the Orono, Me., greenhouse in the winter of 1920–21*

Series.	Inoculation. Method.	Time.[1]	Inoculated plants, 1920–21. Hills.	Date dug.[2]	Progeny, 1921. Hills.[3]	Mosaic.
						Per cent.
1	Juice rubbed into mutilated leaves over entire plant (Pl. 13, A).	I	3	VIII	13	0
		II	3	VIII	8	38
		III	3	VIII	12	100
		IV	3	VIII	13	7
2	Juice rubbed into mutilated leaves of lower third of stem.	I	3	VIII	13	0
		II	3	VIII	10	40
		III	3	VIII	11	27
		IV	3	VIII	8	0
3	Juice rubbed into mutilated leaves of upper third of stem.	I	3	VIII	11	0
		II	3	VIII	11	82
		III	3	VIII	9	67
		IV	3	VIII	10	0
4	Stem split in lower part and a portion immersed for several days in a vial full of juice (Pl. 14, A).	I	3	VIII	9	0
		II	3	VIII	10	0
		III	3	VIII	12	0
		IV	3	VIII	12	0
5	Stem split in upper part and a portion immersed for several days in a vial full of juice.	I	3	VIII	12	0
		II	3	VIII	11	0
		III	3	VIII	13	0
		IV	3	VIII	12	0
6	Capillary tubes full of juice inserted in lower part of stem.	I	3	VIII	11	27
		II	3	VIII	13	31
		III	3	VIII	11	0
		IV	3	VIII	10	0
7	Capillary tubes full of juice inserted in upper part of stem (Pl. 13, B).	I	3	VIII	9	0
		II	3	VIII	10	0
		III	3	VIII	10	20
		IV	3	VIII	13	0
8	As for Series 1	II	3	V	9	33
		II	3	VI	11	82
		II	3	VII	10	100
		II	3	VIII	11	91
9	As for Series 2	II	3	V	5	0
		II	3	VI	10	0
		II	3	VII	11	0
		II	3	VIII	6	0
10	As for Series 3	II	3	V	9	33
		II	3	VI	11	73
		II	3	VII	9	67
		II	3	VIII	10	0
SI	Sources of inoculum	....	6	....	19	100

[1] Dates of inoculation are designated as I, II, III, and IV, indicating, respectively, the time the plant reached a height of from 10 to 15 cm., the time the first flower-bud cluster was exposed preliminary to the abortive anthesis, the fifteenth day after II, and the thirtieth day after II.
[2] The date of digging was either 10 (V), 20 (VI), 30 (VII), or 40 (VIII) days after II.
[3] Each grown from 1 tuber.

It is seen from Series 4 and 5 of Table XIX that the method in which the stem was split and a part immersed in the juice (Pl. 14, A) was ineffective; from Series 6 and 7, that the inserting of capillary tubes full of juice (Pl. 13, B) was slightly effective; and, from Series 1, 2, 3, 8, 9, and 10, that the leaf-mutilation method was less effective when applied to the lower leaves than when applied to the upper leaves or to all the leaves (Pl. 13, A). With application to the upper leaves or to all (Series 1, 3, 8, 10) the inoculum was most infectious when applied at Stages II or III. The complete ineffectiveness of the stem-immersion method and of the leaf-mutilation method at Stage I, and the comparative ineffectiveness of the capillary-tube method, and of the leaf-mutilation method applied to the lowest leaves (Series 2 and 9), all may be caused by the necessity of introducing the mosaic virus in greater quantity, under certain greenhouse conditions, than was done by these methods. The length of time necessary for infection to reach the tubers is indicated, but will be considered in a later section of this paper.

On December 20, 1920, in the Washington greenhouse, spinach aphids (*Myzus persicae* Sulz.), in the adult stage, were transferred from mosaic potato, variety Bliss Triumph, to caged potato plants, variety Green Mountain, varying in height from 2 to 15 cm. Different numbers of aphids were transferred to 12 series of plants each consisting of four potato plants from a single quartered tuber, so that one untreated control was reserved for three treated plants in each series. A set of six series was treated with aphids numbering 1, 5, or 15 individuals to a plant. The remaining set of six series was treated with aphids numbering 2, 10, or 25 individuals to a plant. When the aphids were transferred but one stalk to a pot was allowed to develop. All the treated plants grew under cages, a separate cage to each pot, during the entire experiment.

Eighteen days after aphids were transferred, examination disclosed that every treated plant was infested in proportion to the number of individuals originally placed upon it; some of the plants treated with 25 aphids showed very decided injury. At this time the aphids were killed with nicotine fumigation. On the plants of the first generation distinct mosaic was observed 27 days after treatment with aphids. With one exception the observations on the second-generation plants confirm those which were made on the vines of the first generation. Data on the vines in the second generation are presented in Table XX.

The results in Table XX indicate that in each of the two groups of tuber units there was more infection as more aphids had been introduced. It is not known why 5 and 15 aphids per plant in the 1–5–15 group produced less infection, respectively, than 2 and 10 aphids in the 2–10–25 group, or why in most of the series the same number of aphids did not always transmit the disease. On the whole, this experiment suggests that a larger number of aphids is more effective in transmitting mild mosaic. These results also suggest that very few aphids sometimes are capable of transmitting the disease.

On December 22, 1921, six Green Mountain plants from 6 to 21 cm. in height were inoculated with juice from a rugose mosaic seedling potato. The inoculated plants were kept in the Washington greenhouse under the same conditions as those inoculated by a single treatment with mild mosaic and without being placed in moist chambers. Also the same strain of Green Mountains was used as in the mild mosaic inoculations (p. 48). By January 19, 1922, there was, on five of the

inoculated plants, a dying of the leaves in spots and streaks which frequently was preceded by slight mottling. On the progeny in the second generation, the mottling and uniform wrinkling was usually more pronounced than in the first generation. Leaf spotting and early death of the lower leaves also obtained in the second generation. The controls, in the same tuber units, remained healthy in both generations. Thus another case was added of great contrast between rugose mosaic and mild mosaic.

In the Orono greenhouse during the winter of 1921-22, leaf-mutilation inoculations of four Irish Cobbler hills from two tuber units were made, using inoculum from rugose mosaic Green Mountains. Leaf dropping appeared in all four hills in from 24 to 31 days, but only 7 of the 12 progeny were affected. Parallel inoculations of two Green Mountain hills with inoculum from an Irish Cobbler leaf-roll hill gave negative results in both generations. The results here with two varieties are like those secured in other experiments within either variety.

TABLE XX.—*Effect of variation in the number of aphids upon transmission of mosaic, Washington, D. C., winter of 1920–21*[1]

Series.	Number of aphids introduced.						
	0	1	2	5	10	15	25
31	0:2	....	0:2	....	2:2	....	2:2
32	0:3	....	2:2	....	2:2	....	3:3
33	0:2	....	2:2	....	2:2	....	1:1
34	0:2	....	0:3	....	2:2	....	1:1
35	0:2	0:1	....	2:2	....	0:2	....
37	0:3	0:1	....	0:2	....	1:1	....
38	0:2	0:2	....	0:2	....	0:1	....
39	0:1	1:1	....	0:2	....	2:2	....
40	0:2	0:2	....	0:2	....	1:1	....
41	0:3	....	1:1	....	3:3	....	2:2
42	0:2	0:2	....	1:1	....	0:4	....
43	0:4	....	0:2	....	0:1	....	2:2
Total	0:28	1:9	5:12	3:11	11:12	4:11	11:11
Percentage	0	11	42	27	92	36	100

[1] Progeny of aphid-treated plants given as plants mosaic and total, respectively. Each plant grown from one tuber.

CONCLUSIONS REGARDING INTERVARIETAL TRANSMISSION

The several degeneration diseases described as being diagnosed and transmitted within the Green Mountain variety are also present in other varieties according to the results of intervarietal inoculations. Transmission to Green Mountains from other varieties for ascertaining the identity of the causal virus for various symptom complexes is analogous to the comparison of visible pathogenes from different sources by observing them on the same medium or in the same host. Such comparison indicates that the same virus may induce different symptom complexes in different varieties and that similar symptom complexes in different varieties may be caused by different viruses. Quanjer also points out the value of comparing degeneration diseases in some standard variety

(*39, p. 130*). Some of the differences in diagnosis and terminology may depend upon the use of different standard varieties used in Holland and America, respectively, for such comparison.

INTERSPECIFIC TRANSMISSION OF MOSAIC INVOLVING POTATOES

The definition of mosaic of potatoes, which apparently includes several distinct diseases, has been given (p. 46) and will serve for mosaic of other species of plants. It is not within the scope of this paper to give a review of the extensive literature on interspecific relationships of mosaic. It is sufficient to state here that many species have mosaic, that interspecific transmission from one taxonomic family to another is unusual in comparison with that within such families, and that the mosaic diseases of various hosts, even in the same family, are by no means similar in behavior in regard to seed transmission, infectiousness, viability of the virus, and efficacy of different methods of inoculation. Experiments upon interspecific transmission of mosaic to and from the potato have interest and importance. They indicate the relationship of potato mosaic to other types of mosaic that are better understood and so indicate by analogy the most promising improvements in control measures. They also furnish evidence upon the problem of alternate hosts which might serve to perpetuate and spread infection.

The great variation in mosaic behavior indicates that in attempts at interspecific transmission, the methods used must be selected or modified to suit both species involved. With a given mosaic virus it is desirable to use both a method known to be successful in infecting the usual host and any methods that have been proved effective for a presumably different mosaic virus of the possible alternate host to be tested. In this way the experiment will take into account not only the possibility that the first virus is infectious to the alternate host, but also the possibility that it is infectious only in conditions different from those governing successful inoculation of the normal host.

For example, if needle inoculation alone is used to transmit mosaic from tobacco to potato with observations ceasing in 15 days, it thereby apparently is assumed that the tobacco mosaic virus must act the same in all hosts. On the other hand, if it is deemed possible either that the tobacco virus may be responded to differently in the potato, or even that both mosaics are caused by the same virus with different behavior in different hosts, it is desirable to use methods that are reliable for both mosaics in the respective normal hosts. Another example is a needle inoculation of cucumbers with juice from mosaic potatoes, with observations ending in 13 days. This method may test sufficiently the possibility of the potato mosaic virus being the same as the cucumber mosaic virus, but it would ignore the possibility that the potato virus could be transmitted to cucumbers in the same conditions needed for successful inoculation within the normal host, the potato. Whenever interspecific transmission between two hosts is demonstrated it is still necessary to make further comparisons in regard to symptoms, susceptibility of hosts, ease of inoculation, and incubation period, in order to determine as nearly as possible whether one or more viruses are involved.

POTATOES AND OTHER MEMBERS OF THE SOLANACEAE

In view of the requirements stated above, no completely adequate test has been reported showing that interspecific transmission between pota-

toes and any other members of the Solanaceae is impossible, except the earlier grafting experiments of Quanjer (*38, p. 42*) with tobacco and potato. Transmission of mosaic from tomato to potato and from tomato to tobacco, and the reverse of each, has been reported by Quanjer (*38, p. 42*). Here it seems possible either that a virus was involved, different from that of tobacco mosaic and those of potato mosaic in being infectious to all three hosts, or that the tomatoes were affected only with a combination of tobacco mosaic and potato mosaic. Later, Quanjer has reported transmission of common mosaic by grafting, from potato both to tomato and to tobacco (*39, p. 134*):

> True infectious mosaic ...... can be transmitted by grafting to other solanaceous plants, e. g., tomato and tobacco.

The details of his evidence are not given. Transmission of mosaic from a perennial weed, *Physalis longifolia* Nutt., to potatoes has been reported by Melhus (*27*). Quanjer has indicated the communicability of several degeneration diseases of potatoes to a considerable number of species of the Solanaceae (*39, p. 132, 134, 136*). The experiments to be reported here are with tobacco (*Nicotiana tabacum* L.), tomato (*Lycopersicum esculentum* Mill.), and common nightshade (*Solanum nigrum* L.).

POTATO, TOBACCO, AND TOMATO

Tobacco mosaic can be transmitted readily by means of a needle or by contact which breaks the trichomes (*3, p. 615–17*). The incubation period may be only 5 days (*3, p. 615*) and usually is no longer than 12 to 15 days (*2, p. 17*). Potato mosaic of the most virulent type has not been transmitted by inoculations approaching the needle method in mildness, and has a minimum incubation period of 12 days. With mild mosaic of potatoes unless inoculation is performed early and the growth period of the potato vines is lengthened by growing them either in a field insect cage or in the greenhouse, any infection that occurs will not assuredly be apparent during the first generation. Either tobacco mosaic is caused by a more virulent contagium than any potato mosaic yet described, or, if its cause is identical with that of a potato mosaic, the symptoms are greatly modified by potatoes.

Experiments performed in the Orono greenhouse in the winter of 1919–20 are described in Table XXI. The tobacco plants grew from Connecticut Broadleaf seed, and mosaic was introduced both from dried mosaic tobacco leaves collected by Dr. W. J. Morse in Connecticut and from living mosaic tobacco plants sent from the greenhouse at Washington, D. C. The potato plants were of the Green Mountain variety and came from northeastern Maine.

The data in Table XXI show that methods of inoculation adequate to transmit tobacco mosaic or potato mild mosaic within the species effected no apparent interspecific transmission. Leaf-mutilation juice transfer transmitted mosaic from diseased to healthy potato (Series 2 and 13) and from diseased to healthy tobacco (Series 6) as shown in Plate 15, B, but not from diseased potato to healthy tobacco (Series 1 and 12) or from diseased tobacco to healthy potato (Series 5 and 14). Spinach aphids transmitted mosaic from diseased to healthy potato (Series 4 and 8), but not from diseased potato to healthy tobacco (Series 3) or from diseased tobacco to healthy potato (Series 7). The aphid colonies did not thrive on tobacco sufficiently for control aphid inoculations (diseased to healthy tobacco) to be made but Allard (*3, p. 626–27*)

found this species to be a transmitting agent. It will be noted (Series 9) that aphids from potato inoculated with tobacco mosaic were non-infectious, indicating that potato was not a susceptible masking host or symptomless carrier. Aphid colonies did not establish themselves on the tobacco plants inoculated with potato mosaic, for a corresponding test of tobacco as a symptomless carrier of potato mosaic. A number of control inoculations from healthy plants of one species to the same in the other were made, but are of no significance in view of the negative results from mosaic inoculations.

TABLE XXI.—*Mosaic inoculations between potato and tobacco (Orono, Me., winter of 1919–20)*

Series.	Source of inoculum.	Method of inoculation.	Inoculated plants.
1	Mosaic potato......	Juice stabbed in Feb. 28; leaf mutilation Mar. 9, 15, and 24.	Five, tobacco, largest leaf 10 cm. long Feb. 28. Healthy Apr. 26.
2	...do..............	Leaf mutilation Mar. 6–9, 15, and 24.	Two, potato, 2 cm. tall Mar. 6–9. Healthy Apr. 26. Second generation, 8 of 9 tuber units mosaic.
3	...do..............	Spinach aphids, 25 to 100 to a plant, Feb. 5 or 17. Fumigated 5 to 7 days later.	Ten, tobacco, with 3 to 6 leaves over 1 cm. long when inoculated. Healthy Mar. 26.
4	...do..............	Spinach aphids, 60 to 200 to a plant, Jan. 16 or Feb. 3.	Two, potato, 8 cm. tall when inoculated. Mosaic in 29 to 32 days. Second generation mosaic.
5	Mosaic tobacco....	Leaf mutilation Mar. 5–9, 16, and 24.	Four, potato, 2 cm. tall Mar. 5–9. Healthy Apr. 26. Second generation healthy.
6	...do..............	Leaf mutilation Feb. 16.	Five, tobacco, with 6 or 7 leaves over 1 cm. long when inoculated. Mosaic Mar. 2 and possibly before (Pl. 15, B).
7	...do..............	Spinach aphids, 10 to 80 to a half-tuber, Apr. 14. Fumigated Apr. 21.	Eight, potato half-tubers, with sprouts 2 to 5 mm. long. Plants healthy.
8	Mosaic potato......	Spinach aphids, 25 to 100 to a half-tuber, Apr. 14. Fumigated Apr. 21.	Seven, potato half-tubers, with sprouts 2 to 5 mm. long; 4 of the 7 plants mosaic by June 26, and 5 by July 21.
9	Inoculated potato, of Series 5.	Spinach aphids Apr. 3...	Tobacco, with leaves not over 2 cm. long. Healthy Apr. 26.
10	None..............	None..................	Control parts of tuber units of Series 2, 4, 5, 7, and 8 healthy. Second generation of Series 2, 4, and 5 also healthy.
11	....do..............	....do..................	Control tobacco, healthy Apr. 2. (See Series 6.)
12	As for Series 1......	Leaf mutilation once....	Tobacco, healthy (Pl. 15, B).
13	As for Series 2......	....do..................	Potato. Healthy in first generation. Mosaic in 8 per cent of the second.
14	As for Series 5......	....do..................	Potato, healthy.
15	Mosaic potato	Growth in soil recently occupied by diseased plant.	Many seedlings in 5 pots, healthy 6 weeks after germination.

During the winter of 1919–20 in the greenhouse at Washington, D. C., cross inoculations by leaf mutilation with mosaic between potato, tobacco, and tomato indicated that the tomato was susceptible to potato mosaic, that the tobacco was susceptible to tomato mosaic but not to potato mosaic, and that the potato was not susceptible to tobacco mosaic. These reactions suggested that the tomato either responded symptomatically to two different mosaic diseases or served as a necessary intermediate host for one mosaic between potato and tobacco and vice versa. Accordingly, in the winter of 1921–22 additional cross inoculations between these hosts were made. In addition, cross inoculations with different types of potato mosaic were performed largely between potato and tomato in order to observe the reaction of the tomato to these diseases. The varieties used are indicated in Table XXII. Potato and tobacco plants at the time of inoculation varied in height from 8 to 15 cm. and the tomato plants from 15 to 30 cm. Inoculations in each case were made by leaf mutilation. A single inoculation was made with the exception of Series 15, which received four applications at weekly intervals. Inoculated and control plants came from seed pieces from the same tuber in the potato, and in the tomato cuttings and seedlings from the same lot. Inoculated plants and their controls grew in the same greenhouse, which was fumigated regularly for the control of aphids and white fly (*Aleyrodes vaporariorum* Westw.). The controls remained free from mosaic.

The results shown in Table XXII indicate that the tomato is susceptible to tobacco and potato mosaic and that the symptoms vary between tobacco and potato mosaic, being filiform in part as a result of mosaic tobacco, and mild mosaic or simply mottling, ruffling, and wrinkling as a result of infection with mild mosaic of potato.

Furthermore, the tomato reacted differently to the different types of degeneration diseases represented in the potato, being mild mosaic after inoculation with potato mild mosaic, and rugose mosaic with either rugose mosaic or with streaking plus mottling of potato. Tomato in Series 10 showed the mottling of potato mosaic more readily than the potato itself; mosaic mottling in this case appeared in 12 days, while the potato had failed to show mottling at the end of 27 days.

A comparison of Series 1, 3, and 15 discloses an apparent difference between potato mosaic and tobacco mosaic and suggests the harboring of two distinct mosaic diseases by the tomato. The mosaic tomato in Series 3 became infected from some unknown source, presumably by the potato mosaic in a separate greenhouse during the summer, while the tomato in Series 15 was inoculated with tobacco mosaic, which was not transmitted to the potato in spite of four repeated treatments, as the first and also second generation plants indicated.

TABLE XXII.—*Interspecific inoculations with mild mosaic, rugose mosaic, and streak; greenhouse, Washington, D. C., 1921–22*

Inoculation series.	Date of inoculation.	Source of inoculum.		Inoculated plants.			
		Plant.[1]	Symptoms.	Plant.	Number.	Number infected.	Symptoms.
	1921.						
1	Dec. 13	Tobacco (Connecticut Broadleaf).	Mosaic	Tomato (Norduke)	12	12	Filiform leaves followed by mottling on leaves formed later.
2	Dec. 16	Green Mountain	Mild mosaic	do	16	16	Mild mosaic.
3	Dec. 20	Tomato (Norduke)	Mosaic	Green Mountain	6	3	No mosaic in first generation; mild mosaic in second generation.
4	Dec. 22	Seedling 40552	Rugose mosaic	Tomato (Norduke)	5	5	Rugose mosaic.
5	Dec. 24	Seedling 13858	Streak	do	5	5	Wrinkling and ruffling of leaves.
	1922.						
6	Jan. 4	Green Mountain	Rugose mosaic	do	3	3	Rugose mosaic.
9	Jan. 11	Seedling 41034	Necrotic spotting on leaves	do	4	4	Do.
10	Jan. 16	Green Mountain	No mosaic mottling, although inoculated 30 days ago with mild mosaic juice from Green Mountain. Showed mild mosaic later.	do	5	5	Mild mosaic.
11	Jan. 19	Seedling 41069	Streak and mottling	do	4	4	Rugose mosaic.
14	Jan. 27	Green Mountain	Mild mosaic	do	8	8	Mild mosaic.
[2] 15	Jan. 31	Tomato (Norduke)	Filiform leaves and mottling (inoculated with mosaic tobacco).	Green Mountain	9	0	No mosaic in first or second generation.

[1] Potato, unless otherwise stated.

[2] Four inoculations at weekly intervals.

POTATO AND NIGHTSHADE

Seed from a volunteer plant of common nightshade was planted in steam-sterilized soil in the greenhouse at Orono in the winter of 1919–20. The seedlings were transferred to similar soil in small pots and grouped in five series, as follows.

Series 1

Spinach aphids were transferred from mild mosaic Green Mountain potato plants to 13 caged nightshade plants growing in nine pots and were killed a week later by fumigation. The results, together with other data, are given in Table XXIII. Table XXIII shows that 2 of the 13 nightshade plants were certainly mosaic in appearance and 7 others could be placed almost in the same class (Pl. 15, D). The only plants that showed no mosaic symptoms were 3 that were smaller, when the aphids were introduced, than others in the same pots. Probably they were not fed upon by the aphids. The first mosaic symptom, leaf curling, appeared in from 20 to 47 days after inoculation.

TABLE XXIII.—*Transference of aphids from mosaic potato plants to healthy nightshade plants*

Pot No.	Aphids introduced.		Number of leaves on plant.[2]	Date of appearance of mosaic symptoms.			
	Number.[1]	Date.		Leaf curl.	Leaf collapse.	Wrinkling.	Complete.
1	70	Feb. 17	7	Mar. 16	Mar. 25	Mar. 31	Apr. 6.
			2	None.....	None.....	None.....	
2	40	...do.....	6	Mar. 16	...do.....	...do.....	
3	50	...do.....	7	...do.....	Mar. 25	Mar. 31	Not Apr. 26.[3]
4	60	Feb. 24	9	...do.....	...do.....	Apr. 6	Do.
5	95	...do.....	8	Mar. 31	Mar. 31	Mar. 31	Apr. 12.
6	80	...do.....	7	Apr. 6	None.....	Apr. 6	Not Apr. 26.[3]
			4	None.....	...do.....	None.....	
			2	...do.....	...do.....	...do.....	
7	50	...do.....	8	Mar. 25	Mar. 25	Mar. 31	Do.[3]
8	80	...do.....	7	Mar. 16	Mar. 31	Apr. 6	Do.[3]
9	80	...do.....	7	Mar. 25	Mar. 25	...do.....	Do.[3]
			5	Apr. 12	None.....	Apr. 12	Do.[3]

[1] Approximate.
[2] Over 1 cm. in length, including the cotyledons.
[3] Probably mosaic, however, even though not completely so.

Series 2

Healthy controls to Series 1, with each of five plants in a separate pot, were fed upon for a week by 40 to 100 aphids. The aphids were introduced on March 8 when there were eight or nine leaves each over 1 cm. in length. No mosaic symptoms had appeared on April 26, when all plants were discarded, in marked contrast to those of Series 1 growing alongside (Pl. 15, D).

Series 3

On February 9, 1920, juice expressed from mild mosaic potato plants was inoculated into the mutilated leaves of 10 nightshade plants when the number of leaves over 1 cm. long was from three to eight. Only

leaves of this length were inoculated, and these were so thin they could not be bruised as much as is necessary for the successful inoculation of potatoes. They were somewhat mutilated with the finger nails and by pinching, with juice present on the fingers and applied after mutilation. One of this series became mosaic (Pl. 15, D).

Series 4

Healthy controls to Series 3, with each of five plants in as many pots, were inoculated similarly with juice from healthy potato plants. No mosaic symptoms appeared by April 26.

Series 5

Three untreated controls were transplanted and 13 were not. All remained healthy throughout the experiment.

The five preceding series indicate that potato mild mosaic virus is infectious to a high degree when introduced by aphids, and to a slight extent when introduced by leaf mutilation, to nightshade. Return inoculations from mosaic nightshade plants to potatoes were made to sprouted tubers in the same manner as in the tobacco-potato Series 7 and 8 of Table XXI. It will be remembered that in the control Series 8, aphids from mosaic potato plants infected 71 per cent of the half-tubers. The 29 per cent of Series 8 that remained healthy were fed upon by fewer aphids than the rest of the series, only 30 being introduced to a half tuber. Two series were involved with nightshade and sprouted potato tubers, as described in the following paragraph.

Series 6

Spinach aphids on mosaic nightshade plants were not very numerous. Those present were on the plant of pot 8, Table XXIII, under a cage, and were established there on April 6 as a proved nonvirulent colony from radish plants. Fifteen or 20 were transferred to each of five sprouted half-tubers and 20 per cent of the half-tubers became mosaic. Thus mosaic was transmitted from nightshade to potato tubers by fewer aphids than were required to transmit it from mosaic potato vines.

Series 7

Healthy controls to Series 6 were fed upon by aphids from healthy nightshade plants, which produced no infection.

Series 6 and 7 indicate that if enough aphids are transferred to potato from mosaic nightshade, infected from potato, there will be as much infection as when they are transferred from mosaic potato.

The experiments with nightshade and tobacco were performed in the same greenhouse but in different rooms. Since tobacco mosaic can be transmitted to nightshade (*2, p. 10*) it was slightly possible that the mosaic nightshade plants had become infected in some way from mosaic tobacco. Therefore spinach aphids were transferred from a mosaic nightshade plant to small tobacco seedlings, on April 6. The aphids fed and increased until April 26, but all the tobacco seedlings remained healthy.

POTATOES AND PLANTS IN DIFFERENT FAMILIES

Transmission of mosaic from cucumbers (*Cucumis sativus* L.) to potatoes has been reported by Doolittle and Walker (*11*). The mosaic of cucurbits seems to be the most virulent type known and to be the type most easily transmitted from one family of hosts to another. However, as in northeastern Maine, where potato mosaic is common, the writers have not yet found any wild members of the Solanaceae, it has been necessary to consider the possibility of transmission of potato mosaic between families in connection with the problem of perpetuation in weeds. In this region the writers have observed mosaic in garden beans (*Phaseolus vulgaris* L.), evidently introduced in the seed. A very common mosaic is that of the numerous wild red raspberries (*Rubus* sp.). A mosaiclike disease is sometimes common on certain composites and resembles one type of aster yellows. The raspberry mosaic is the only one of these diseases that has been tested by the writers in regard to transmissibility to potatoes.

POTATO AND RASPBERRY

In the Orono greenhouse in the winter of 1920–21, cultivated mosaic raspberry bushes were transplanted in October and grown until the period of dormancy was passed. On January 28, the new shoots were ground up and juice obtained consisting of distilled water and what could be washed and squeezed out of the rather dry pulp into the water. The juice was used in leaf-mutilation and capillary-tube inoculations corresponding to those described in Table XIX, where potato mosaic was transmitted. Each method was used in four hills taken, respectively, from four 4-hill tuber units. The 16 plants in these tuber units were all healthy, as were also the 50 plants of the second generation. This indicates that two methods of inoculation that transmitted mosaic from potato to potato, were not effective in transmitting mosaic from raspberry to potato. No aphids abundant in northeastern Maine are known to infest both Rubus and potato (*37*), which might explain why proximity of healthy potatoes to mosaic raspberry has caused no apparent infection.

CONTROL OF DEGENERATION DISEASES OF POTATOES

The desirability of controlling any one or more of the degeneration diseases depends upon the effect on the yield rate and on the quality. The determination of such effects requires correct diagnosis of the disease or diseases involved. With a given disease, distinction should be made between hills or plots with current-season infection (usually no symptoms) with second-season infection (usually first-season symptoms) and with third-season infection (Pl. 14, B). Until it is clearly shown that disease-free strains (stocks of the same variety recently secured from different sources) have no inherent differences that survive growth and the production of seed in the same environment, it is also desirable to compare effects of a disease in parts of the same strain. It may be readily seen that it is difficult to secure an extensive comparison fulfilling the preceding requirements and giving due consideration to soil, variety, and climate or season, especially when infectious diseases are present to contaminate the healthy parts of strains. Further, the total percentage of incidence may affect the average reduction of yield rate by each per cent of diseased hills, and diseases in combination may have an effect

greater or less than the sum of the effects of both considered separately. With a given yield rate, the quality may be affected with a loss depending somewhat on the demands of the trade regarding the characteristics of the tubers or progeny.

ADVANTAGES TO BE DERIVED FROM CONTROL

Table XXIV summarizes the results of a number of yield tests that have been made with parts of the same strain in each of two varieties. The L strains secured on the L farm will be discussed first. In 1918, on Aroostook Farm, the yield rates were reduced by an all-mosaic condition (mild type) slightly more for Bliss Triumphs than for Green Mountains in comparison with plots with part of the hills—the mosaic ones—removed during the season. In 1919, on Aroostook Farm, the yield rates were reduced by an all-mosaic condition much more for Bliss Triumphs than for Green Mountains, in comparison with plots with about a fifth of the hills mosaic. In 1920, on a field with poor soil and cultural conditions, the yield rates were reduced less by an all-mosaic condition for Bliss Triumphs than for Green Mountains, in comparison with plots with about a fifth of the hills mosaic. In 1919, the only year of the three in which conditions were at all normal and favorable for growing potatoes and for making such a test, for each 10 per cent of mosaic the reduction amounted to 2.8 barrels [4] an acre for Green Mountains and to 5 barrels for Bliss Triumphs.

In these two strains and in many small lots (*40, p. 316*) the writers have not observed an increase in severity in symptoms except in some cases where another disease, such as the spindling-tuber disease, came in or increased, as in the following tests. In 1921, on Aroostook Farm, the same Green Mountain stock mentioned above was again used for yield tests. One part about a third mild mosaic and a third spindling tuber, both first-year symptoms following infection late in 1920, had a yield rate of 146 barrels an acre. A part all mild mosaic, as for several years at least, and about a third spindling tuber (first-year symptoms) had a yield rate of 96 barrels. Another part all mild mosaic, as for several years, and all spindling tuber with the second-year symptoms (third year of disease) had a yield rate of 68 barrels.

Comparing the first and second parts gives a reduction of about 7 barrels an acre for each 10 per cent of mild mosaic, while comparing the second and third parts gives a reduction of about 4 barrels an acre for each 10 per cent of spindling-tuber disease in mild mosaic stock. In these tests the size of the plots varied from one-fifth to one-fourth of an acre in 1918 and 1919, from one-ninth to one-fifth in 1920, and from one-eleventh to one-sixteenth in 1921.

Further comparisons in Table XXIV lead to the following conclusions: The mosaic part of a strain sometimes yielded less than a partly rogued healthy part, as in Green Mountain strain L (1918, 1920), in Bliss Triumph strain L (1918, 1919, and 1920), and in Green Mountain strain S (1920). The mosaic part of a strain sometimes yielded more than a rogued healthy part but only so if the allowance is not made for loss by roguing, as in Green Mountain strain L (1919), strain S (1918 and 1919), and strain W. The mosaic part of a strain sometimes yielded less than an unrogued healthy part, as in Green Mountain strain L (1919; 1920 on P farm;

[4] A "barrel" (barrelful) is 165 pounds, or 2¾ bushels.

1920 on Long Island; 1921), Bliss Triumph strain L (1919; 1920 on P farm), and Bliss Triumph strain R. In one case (Green Mountain strain C) a slightly mosaic part yielded a little better than a no-mosaic part. The spindling-tuber disease decreased the yield rate where present in Green Mountain strain L (1920 and 1921). In this strain growth on Long Island was accompanied by a greater reduction in yield rate by mosaic than on P farm.

TABLE XXIV.—*Yield tests on effect of mild mosaic and spindling-tuber disease within strains*

Strain.[1]	Year.	Part.	Area.[2]	Yield rate.	
				Barrels per acre.	Pounds per hill.
			Acre.		
Green Mountain L.	1918	Rogued of 11 per cent mosaic..	1/5	89	1.35
		All mosaic..................	1/4	69	.93
	1919	Rogued of 20 per cent mosaic...	1/4	[3] 104 (130)	....
		20 per cent mosaic...........	1/5	144	....
		All mosaic..................	1/5	122	....
	1920	Rogued of 15 per cent mosaic..	1/10	....	1.97
		Rogued of 21 per cent mosaic..	1/10	....	2.00
		21 per cent mosaic, on P farm..	1/6	83	1.09
		50 per cent mosaic...........	1/10	....	1.11
		12 per cent mosaic, all spindling tuber.	1/30	....	.97
		All mosaic on P farm.........	1/5	65	.78
		16 per cent mosaic on Long Island.	1/11	....	1.91
		All mosaic on Long Island....	1/15	....	.58
	1921	36 per cent mosaic and about 30 per cent spindling tuber.	1/5	146	....
		All mosaic and about 30 per cent spindling tuber.	1/16	96	....
		All mosaic and all spindling tuber.	1/8	68	....
Bliss Triumph L.	1918	Rogued of 15 per cent mosaic..	1/5	75	1.22
		All mosaic..................	1/5	53	.82
	1919	Rogued of 20 per cent mosaic...	1/4	84	....
		20 per cent mosaic...........	1/5	100	....
		All mosaic..................	1/5	60	....
	1920	Rogued of 26 per cent mosaic...	1/10	....	1.34
		44 per cent mosaic...........	1/10	....	1.14
		21 per cent mosaic on P farm...	1/5	84	1.17
		All mosaic on P farm.........	1/9	82	1.09
Green Mountain S.	1918	Rogued of 13 per cent mosaic.	1/4	[3] 84 (97)	1.31
		45 per cent mosaic...........	1/2	86	1.10
	1919	Rogued of 30 per cent mosaic...	1/4	[3] 112 (160)	....
		68 per cent mosaic...........	1/4	133	....
	1920	Rogued of 22 per cent mosaic..	1/10	....	1.79
		89 per cent mosaic...........	1/10	....	1.75
Green Mountain W.	1919	Rogued of 32 per cent mosaic...	1/4	[3] 106 (156)	....
		30 per cent mosaic...........	1/4	147	....
Green Mountain C.	1920	0.2 per cent mosaic...........	1/10	....	1.47
		6 per cent mosaic............	1/10	....	1.55
Bliss Triumph R.	1920	Rogued of 2 per cent mosaic...	1/10	....	1.69
		7 per cent mosaic............	1/10	....	1.26

[1] Each strain was divided in the year previous to the first given here, by hill selection or by part being rogued.
[2] Area given is approximate and on Aroostook Farm unless otherwise stated.
[3] Yield rate in parentheses is that calculated for 100 per cent assuming the rogued percentage to have been a directly proportional loss.

Mosaic is well distributed over the United States (*45, p. 248*; *14, p. 158*), having been reported in 1917 and 1918, from 21 States, including all those in which potatoes are an important crop. The existence of several types and the modification of symptoms by varietal and environmental factors make it probable that the true extent and meaning of the geographical distribution of mosaic is merely beginning to be fully realized. Sixty-eight strains of Green Mountains from seed grown in Canada, Maine, New York, Vermont, and Wisconsin, were tested in one place and found to have an average of 33 per cent mosaic (*1, p. 11*) and as high as 48 per cent (*35, p.* 8). Mosaic was common in seed-potato fields of Maine and northern New York in 1915 (*46, p. 357*). The average mosaic percentage for Green Mountains in New York and Aroostook County, Me., is given by Barrus (*6, p. 13*) as 50 per cent or more, with 20 per cent or less as rare. During 1919, the writers made a careful estimate of the amount of mosaic in 40 Green Mountain fields and the same number of Bliss Triumph fields in northeastern Maine. Many of these were supposed to be above the average in freedom from the disease. Mosaic varied from one-half per cent to 100 per cent, averaging 28 for the Mountains and 46 for the Bliss. Similar results followed the examination of fewer fields of these varieties in 1920, and 20 supposedly choice American Giant fields were found to contain from 3 to 46 per cent, averaging 13. In Green Mountains in 1921, 26 fields in northeastern Maine averaged 32 per cent, and 58 fields in New Brunswick, mostly in the northern part, averaged 13 per cent mosaic. Most of the mosaic in commercial fields in northeastern Maine and New Brunswick is of the mild type. Variation in Louisiana from 3 to 90 per cent in Bliss Triumps is reported (*12, p. 8*), with losses as high as total failure (*12, p. 3*).

The reduction of yield rate by leaf roll has not been determined in plots. Leaf roll does not extend so far north as a prevalent disease as does mosaic. Both are prevalent in western New York and southern Ontario (*29, p. 36*), while in northern Ontario mosaic is troublesome but leaf roll is not (*28, p. 5*). The writers have found leaf roll much less common than mosaic in northeastern Maine in varieties badly affected elsewhere with both (*41, p. 75*). When leaf roll causes net necrosis in the tubers, the quality of the crop is affected as well as the yield.

Leaf-rolling mosaic alone appears to affect the plants about as mild mosaic does, but in combination with spindling-tuber disease (giving a form of curly dwarf in Green Mountains as least) the yield rate is considerably reduced (*46*).

The spindling-tuber disease seems to reduce the yield rate less by the first-year symptoms (in plants growing from normal shaped tubers produced by plants infected late in the season) than by the second-year symptoms (in plants growing from spindling tubers) (Pl. 14, B). It is present in all percentages of incidence in several varieties in northeastern Maine. Its presence in at least 10 widely separated States is known from personal observations by the writers and from personal and written reports by pathologists. Its production of abnormally shaped tubers injures the quality of the crop for sale as seed and table stock.

Rugose mosaic, streak, and unmottled curly dwarf are each more injurious to the yield rate than the four diseases just mentioned. They, and also leaf-rolling mosaic and spindling-tuber disease, are probably

more generally distributed over the potato-growing regions than is shown by reports now available.

Lack of control of these various degeneration diseases causes losses to seed buyers because of their appearance in plants grown from apparently normal and healthy tubers. This reacts on the sellers of seed. Control therefore will not only improve the yield of generally salable tubers, but will also improve the quality of the crop for seed purposes.

GENERAL FACTORS INVOLVED IN CONTROL

TRANSMISSION

From the results of the experiments described in preceding parts of this paper, it may be concluded that by aphids and possibly other means there is danger of degeneration diseases spreading in the field from diseased to healthy hills or from diseased to healthy fields. A stock of Norcross (Green Mountain group) grown in a large field on Aroostook Farm had 55, 80, 95, 99, and 100 per cent of the hills mild mosaic, respectively, in the five years from 1918 to 1922. Other stocks of smaller size have here shown a similar steady increase of mild mosaic and of spindling-tuber disease, but not of leaf roll. That such frequent annual increase in the percentage of incidence is due to infection of healthy hills from the diseased ones in the same field is shown by certain hill selections, to be described in a later section of this paper. Also, the effect of different degrees of isolation of healthy stocks from diseased stocks will be shown in a later part of this paper. The removal of diseased hills serving as sources of infection, and the control of insects that transmit these diseases, are important phases of control to be given attention later.

If any experiment should eventually indicate that transmission through the soil is possible, it will be necessary to test or eliminate various soil factors (such as subterranean insects, soil water, soil mass, or débris from diseased plants) in order to determine the exact method of transmission. At present neither soil nor root-contact transmission has been demonstrated.

PERPETUATION

The question of perpetuation in diseased weeds has been considered in connection with interspecific transmission. It seems to be of importance in some regions and not in others.

The possibility of perpetuation in the soil exclusive of diseased tubers has been tested in pots, with negative results in both the first and second generations (*40, p. 335*). This experiment involved contact with some of the roots of plants recently removed. Field experiments also gave negative results with mosaic, both in the first (*40, p. 335–36*) and second generations, and whether begun upon the ground of 1918 plots or of 1919 plots. In the latter (Table XXVII, field D) dead mosaic vines were buried in the furrow at planting time. The same negative results resulted from tests with leaf roll reported by the writers (*41, p. 59*) by Quanjer (*38, p. 41–42*), Wortley (*50*), and Murphy (*29, p. 52–53*) and from tests with mosaic dwarf by Krantz and Bisby (*21, p. 15–18*).

The well-known perpetuation through the tubers is of great importance. It requires the avoidance of fields containing viable overwintering diseased tubers such as were present in northeastern Maine in the spring of 1919 (*40, p. 335*) and of 1921 (Pl. 14, C).

It makes it desirable to inspect the fields that produce the seed tubers, since the condition of the parent plants has more effect on the progeny than the conditions of seed-tuber storage or of the growth of the progeny. Other phases of tuber perpetuation will be discussed in connection with the question of tuber selection.

DIFFUSION OF THE CONTAGIUM

The spread of the contagium or virus within the plant will be termed "diffusion." Stocks assuredly mosaic, that have shown the symptoms of mild mosaic for several seasons, usually do not show the symptoms in the first part of the season (several days to several weeks) and often lose them in the last part. This seasonal cycle is considered by the writers to be the result not of diffusion of the contagium to and from the leaves, but rather of the effect of the degree of maturity upon leaves containing the virus, and frequently shows variations due to temperature and other environmental factors. Diffusion of the virus from the point of inoculation in the vines to the tubers may occur without the appearance of symptoms, even after the normal period of incubation, if the leaves have ceased growing. The time needed for this diffusion is of importance in relation to the possibility of tuber infection being reduced by an early death of the aerial parts of the plants, after the latter have been inoculated by insects.

Knowledge of virus diffusion may explain the disease division of hill lots, tuber units, hills, and shoots, where the progeny of a hill or of a tuber, and even a single shoot, may be only partly diseased. Although such disease division may be caused by recent insect inoculation in some cases at least, it occurs in conditions where such insect inoculation is not a satisfactory explanation. Incompleteness of diffusion is of interest to those who test hills or tubers by sampling one eye of each, and to those who plant a seed plot by tuber units. If its occurrence in a seed tuber is sometimes the cause of the late appearance of symptoms in the upper part of the plant, it is also important to those who attempt to remove all diseased hills early in the season.

In the winter of 1920–21, at Orono, the plants of nine tuber units were inoculated at different times by the leaf-mutilation method with inoculum from mild-mosaic Bliss Triumph plants, and were dug within a certain period of time after inoculation. Three different intervals elapsed for parts of each tuber unit. The progeny were grown in the field in 1921, and showed symptoms as described in Table XXV, for Series 1. In another series (No. 2 of Table XXV) the plants of three tuber units were inoculated with a reliable method similarly at a given time and four different intervals elapsed for parts of each tuber unit. In each series, 10 days allowed much less diffusion of the virus to the tubers than 20 days or longer. This helps to explain why digging seed tubers within 10 days after the maximum infestation by transmitting insects avoided the maximum amount of disease (p. 104). It also indicates how early autumnal killing frosts in northern regions, following the usual late spring hatching of aphid eggs and consequent late development of infestation, might contribute to the general less abundance of degeneration diseases in the north, resulting in the well-known preference for northern-grown seed (*10, p. 162–63*).

TABLE XXV.—*Effect of difference in time elapsed between leaf-mutilation inoculation with mild mosaic and removal of tubers*

Tuber-unit series.	Inoculation period.	Date of tuber removal (number of days after inoculation).	Progeny mosaic.[1]	
			Number inoculated.	Mosaic.
				Per cent.
[2] 1	Exposure of first flower-bud cluster	40	29	55
	Fifteenth day after bud exposure	25	32	66
	Thirtieth day after bud exposure	10	31	3
[3] 2	Exposure of first flower-bud cluster	10	9	33
		20	11	82
		30	10	100
		40	11	91

[1] 1 hill from each tuber.
[2] Series 1, 2, and 3 of Table XIX, excluding plants inoculated before anthesis.
[3] Series 8 of Table XIX.

It has been reported (*40, p. 318*) that a small proportion of tuber units may contain both healthy and mosaic hills, and that in such mixed tuber units the diseased hills are at first about evenly distributed between bud-end hills and stem-end hills but later are more common in the bud-end hills. The difference thus shown at first between sister hills in regard to mosaic is apparently due either to unequal retardation of the appearance of symptoms or to unequal distribution of the virus in the tuber. The later development of the difference between bud-end and stem-end hills may be due, as far as is known, to the greater number of eyes in the bud-end quarters and the resulting better chance to include a diseased eye, or to field infection that affects the faster-growing bud-end plants more and that produce symptoms apparent the same season.

In some cases of possible early field infection with current-season symptoms, at first only part of a hill or stalk is diseased—either one branch or the upper leaves or even one side of a branch, and the infection then spreads to the new growth, whereas perpetuation by tubers from diseased hills is followed by uniformly distributed symptoms. This partial infection appears later than that which evidently follows tuber perpetuation, being in 1921 about the only type found after the second roguing in a rogued and isolated seed plot that contained aphids earlier in the season. It is correlated with the presence in the field of virus diseases especially of the more injurious and virulent types, being more abundant in the experimental plots than in commercial fields, where it is found infrequently. Furthermore, it is found, in experimental plots, indiscriminately in partly diseased lots, in lots from stock that came from healthy fields and that are healthy in commercial fields, and in lots with the preceding generation grown under cages or in greenhouses with no opportunity or evidence of infection occurring. Murphy has noted such partial display of current-season symptoms (*29, p. 62–63*).

In 1920, several thousand Green Mountain tubers from many sources, mostly experimental plots, were split in two and planted as 2-hill tuber units. Thirty-seven tuber units either were partly mosaic—that is, with one or more stalks partly or wholly healthy, in the first week of July (when progeny of all-mosaic lots planted in the same field in the same

way were all diseased)—or were healthy then and partly mosaic later. In these 37 tuber units, often different stalks in a hill were affected with different severity. Whether healthy or partly mosaic on July 1, these units became more diseased as the month progressed. They could be arranged in a series of gradually increasing amount and severity of disease. They seemed to be either the last hills to show mosaic as the result of 1919 infection or hills that showed the first symptoms resulting from 1920 infection. If the last to show 1919 infection, they would seem to be so because of incompleteness of infection of the tubers in 1919. If they were mosaic as the result of 1920 field infection, the identity of the transmitting agents is in doubt, since aphids were not discovered on June 26. On July 26 they were limited to colonies each of about a dozen consisting of a mature potato aphid individual and her young ones. A shoot in the Orono greenhouse, where aphids were absent, was observed having one side mosaic, the line of distinction between diseased and healthy even bisecting two leaves lengthwise.

Whatever the cause of delayed and partial manifestation of symptoms, the effects are troublesome. About a fourth of these 37 tuber units became affected with streaking between July 1 and 26. This streaking was either accompanied or preceded shortly by the appearance of brittleness and mottling. A few more became thus affected between July 25 and August 27, one alone with no mosaic mottling. This single apparent exception to the rule of association of mottling and streaking was not surprising since mottling had become reduced in distinctness or entirely effaced in over one-third of the tuber units. Thus, in certain tuber units streaking was an accompaniment of mottling that appeared later than normal and that was found in only part of each unit. Moreover, in many of these units mottling became reduced during August even though the variety was Green Mountain, one that retains this symptom better than most varieties, and the streaking was followed in part by progressive necrosis. As a result, a combination of streaking, brittleness, and leaf dropping could be seen in August with no mottling evident in the plants, and were it not for the wrinkling present, such plants would have been considered by anyone without access to previous records as being affected only with streak. Furthermore, at one time it was possible to arrange the streaked hills in a series with complete gradation from a mottled-streaked condition to a typically streak condition. It seems possible that this change and gradation from mosaic to streak was due in part to the presence of two viruses—rugose mosaic and streak, in part to variation in the time of infection (or to variation in diffusion of the virus), and in part to the increase in maturity of the plants. However, in this and similar cases, it is also possible that a single virus (rugose mosaic) produces streaking and leaf dropping in the conditions described. At any rate, correct diagnosis and the early removal of diseased hills seemed difficult under such conditions.

RESPONSE TO ENVIRONMENTAL FACTORS

Environment influences both the symptoms and the rate of spread of degeneration diseases. New symptoms following the transferring of a given lot to another region can not be regarded as the effect of the new environment unless either there is evidence that there was no new infection during the last season in the first region, or a similar part of the stock is kept in the first region as a control. Loss of symptoms following

such translocation may be considered as a result of masking by the new environment, unless they are masked by new symptoms of a second disease.

Tubers of a mosaic lot were divided and part of each tuber was planted in northeastern Maine and part in Colorado (*45, p. 250*). During the same season, mottling was distinct in the part in Maine and absent from the other. A similar lot was divided likewise and grown partly in northeastern Maine and partly at Washington, D. C. (*45, p. 250*), and during the same season mottling was distinct in Maine but doubtfully ascertained in many hills at Washington. In three successive seasons a number of partly mosaic lots were divided and grown partly in northeastern Maine and partly in southwestern Maine (*45, p. 250*). Usually the part of a lot grown in the southwestern area showed much less mottling than the other part, while the reverse never was noted. Recovery of the mottled appearance followed the return of a part to the northeastern area.

In 1922, a similar test on the effect of different climatic conditions on plants from the same tuber was conducted with 30 Bliss Triumph tubers and with an equal number of Green Mountain tubers apparently having mild mosaic. A seed piece from the same tuber was planted in each of these localities: Presque Isle, Me.; Riverhead, Long Island, N. Y.; and Norfolk, Va. Observations were made by the same person, one of the writers, in the three localities. The results of these observations indicated that mottling was less distinct on the foliage at Norfolk than in the other localities. It was also noted that most of the vines in the three localities showed mild mosaic symptoms; a few plants plainly disclosed mosaic-dwarf or bad mosaic symptoms. However, mosaic-dwarf appeared on the plants from the same tuber in each of the three localities and no tuber produced plants having mild mosaic in one locality and a different stage of mosaic in another locality. It appears that the difference in climatic conditions here as in the preceding tests chiefly produced a difference in the distinctness of the mottling, the stage or type of mosaic remaining the same.

Progeny of Green Mountain curly dwarf hills were grown partly in a warm room (approximately 67° F. or 19° C.) in the Orono greenhouse in 1919–20, and partly in a cool room (approximately 56° F. or 13° C.). Mottling appeared, was mostly restricted to the tissues along the veins, and was so restricted more in the warm room. The same was true of progeny of mosaic hills selected in the same lot. Such a hill grown in the open is shown in Plate 15, A.

In 1919 a stock of Green Mountains with about 80 per cent mosaic was grown on a number of fertilizer plots. Both the percentage of mosaic plants and the distinctness of mottling in those mosaic were reduced somewhat during the first part of the season by high-nitrogen and high-potash fertilizers.

In 1919 four mosaic tubers were split in two and one-half of each was planted under an insect cage in the field and the other in the open. In two cases the caged hills were more distinctly mottled than the corresponding uncaged ones.

Twelve tubers from Bliss Triumph mosaic hills were grown in the Orono greenhouse in 1920–21. They were split in two, and one half of each was planted in a warm place (approximately 75° F. or 24° C. mean temperature) and the other half in a cool place (approximately 60° F. or 16° C. mean temperature). Wrinkling and mottling were more marked

in the cool place (Pl. 15, C). When partly grown, the plants in the warm place were put near the others in the cool place. Then the difference failed to continue in the upper parts of the plants, since the leaves that expanded upon the transferred plants were like the corresponding leaves of the plants kept in the cool place from the first. This experiment confirms one of those performed by Freiberg (*16, p. 195–202*), in which potato and other plants showed apparent recovery from mosaic under certain conditions of temperature. Murphy has described the seasonal masking of mosaic symptoms (*30, p. 148*), and Johnson has demonstrated the suppressing effects of temperatures over 20° C. (*19*).

Ten whole tubers from a leaf-roll stock were planted in the Orono greenhouse during the winter of 1921–22. Three of the 10 plants were grown caged, and of these three, two showed no distinct rolling, though all three were chlorotic and burned. The uncaged plants were rolled until after the roof was whitewashed in April, after which new leaves that grew out were only chlorotic and flat. This diminishing of rolling was noted also under field cages in 1921. Such an effect from caging or other means of shading is not surprising in view of the evidence that leafroll is associated with the abnormal accumulation of food, which is caused by the more constant internal symptom of phloêm necrosis (*38, p. 43–45*), probably only in the presence of sufficient sunlight.

In 1921 tubers from hills diseased in 1920 were split in two and the halves of each tuber were planted, respectively, under and outside of insect cages. The disease in 1920 was either streak, curly dwarf, or mosaic, and several varieties, including Green Mountains and Rurals, were used. The summer was warm and dry, so that the cheesecloth cover probably caused changes in environmental factors as shown by Burns for wire-screen cages (*9*). Comparisons were made several times during the season. The hills grown outside of cages usually were smaller than the inclosed sister hills. The shading in the cages enlarges healthy plants so that this difference in diseased plants is not surprising. Such a difference in size is not due to different degrees of dwarfing so much as to the same degree of dwarfing acting upon plants of different sizes. In a few cases mottling was present in the cages while the chlorosis outside was diffused. Symptoms observed in common to these environments were wrinkling (in Green Mountains), ruffling (in Rurals and Green Mountains), brittleness (in certain seedling varieties), burning (in seedlings, Rurals, and Mountains), and premature death (in seedlings). This experiment indicates that a reduction in sunlight may decrease the apparent dwarfing effect of a disease and may increase mottling. It also shows that in certain climatic conditions burning is not prevented by shading or by protection from insects provided certain diseases are present.

In 1920 parts of the same stock of a strain of the Green Mountain variety were planted in two fields in northeastern Maine and in a field on Long Island. The yield rate in one field in Maine was almost double that in the other and but little more than that on Long Island. The mosaic percentage was low. Another part originally of the same strain but all-mosaic was planted in the poor field in Maine and in the Long Island field. Its yield rate was lower on Long Island, even with field conditions twice as good for healthy stock. Thus an all-mosaic condition reduced the yield rate of Green Mountains much more on Long Island than in northeastern Maine. Stewart reports heavy losses upon Long Island (*46, p. 357*). Murphy describes the effects of season, locality, and climate on the yield-rate reduction by leaf roll (*29, p. 40–44*) and mosaic (*29, p. 67*).

In addition to mosaic symptoms being masked or modified by regional differences in weather and climate, there are differences between regions in regard to the amount of degeneration diseases and the rate of their spread in commercial fields. This is shown by local observations and by comparing representative lots from different regions when grown in the same place. Differences are apparent in three groups of lots from three counties of New York (*1, p. 11*). The writers have reported a difference between different parts of Maine with mosaic spreading less in the St. John River Valley where aphids were less numerous (*41, p. 75*). This difference disappeared in 1921 with a heavy infestation of aphids in that valley, as is described in connection with certain data on isolation (Table XXVIII, field 38). Similar regional differences have been described by Murphy (*29, p. 35–36, 59–67*) and Quanjer (*39, p. 143*).

The second-generation plants to the lots grown in Maine and Long Island, as noted in a foregoing paragraph (p. 101), were grown again in Maine, where the chief difference between these lots consisted in a higher percentage of leaf roll in the Long Island stock than in the duplicate Maine lots, which had no leaf roll.

In 1921 a Green Mountain lot grown in Maine in 1920 was divided into five parts, which were planted, respectively, between mosaic Bliss Triumph rows in northeastern Maine and in southern Maine, between Irish Cobbler leaf-roll rows in these two places, and on Long Island between Green Mountain rows both all-mosaic and 25 per cent leaf roll (Table XXVI). In 1922 the Long Island part when grown in northeastern Maine was 60 per cent mosaic and 75 per cent leaf roll, while at the same place the other stocks showed less disease (Table XXVI). Similar exposure to leaf roll and mosaic at the two Maine stations was made in 1921 with a Bliss Triumph lot and an Irish Cobbler lot, and a comparison in 1922 in northeastern Maine showed that a slightly greater spread of mosaic had occurred in northeastern Maine and a markedly greater spread of leaf roll in southern Maine (Table XXVI).

TABLE XXVI.—*Spread of mild mosaic and leaf roll in three places*

Variety.	Location in 1921.	Disease in 1922.	
		Mosaic.	Leaf roll.
		Per cent.	*Per cent.*
Green Mountain	On Long Island between Green Mountain rows 100 per cent mosaic and 25 per cent leaf roll.	62	75
	In southern Maine between mosaic rows	31	0
	In northern Maine between mosaic rows	57	0
	In southern Maine between leaf-roll rows	40	50
	In northern Maine between leaf-roll rows	40	3
Bliss Triumph	In southern Maine between mosaic rows	44	0
	In northern Maine between mosaic rows	56	0
	In southern Maine between leaf-roll rows	26	19
	In northern Maine between leaf-roll rows	55	8
Irish Cobbler	In southern Maine between mosaic rows	0	0
	In northern Maine between mosaic rows	2	0
	In southern Maine between leaf-roll rows	2	14
	In northern Maine between leaf-roll rows	23	4

RESULTS OF TESTS OF CONTROL MEASURES

With the preceding data in mind, tests of various control measures can be made and interpreted more intelligently. Seed treatment seems useless, since the plant juice containing the contagium can not well be poisoned. Heat does not injure the virus before injuring the tuber (*8, 19*). The only apparent solution is the selection of noninfected seed by one or more of the methods which will be discussed.

TUBER SELECTION

The selection of hills and strains is in the broad sense a type of tuber selection, but the term "tuber selection" is used here as meaning the selection of tubers in the bin without knowledge as to the health of the parent plant. The elimination of tubers with net necrosis of a certain type will reduce the amount of severe leaf roll in the progeny (*41*). Discarding tubers with the spindling-tuber symptoms (produced by plants in the second year of infection) will reduce the amount of third-year infection of the spindling-tuber and unmottled curly-dwarf diseases. (First-year infection in field occurs late and does not show in vines or tubers.) The use of only the largest potatoes will reduce the amount of third-year infection of mosaic and leaf roll (*41, p. 76–77*). However, there will probably always be tubers of good size and shape that were produced by plants infected the previous year or before and that are perpetuating disease, unless the field producing the crop was free from infection.

With the spindling-tuber disease it is possible to eliminate a large percentage of the spindle-shaped or "run long" tubers and so reduce the percentage of this disease. However, tuber selection alone, even with the spindling-tuber disease, does not necessarily result in eliminating this malady, since many of the normal-shaped tubers will be infected, due to late season transmission by aphids in the field. Also, symptoms are not always conspicuous (Pl. 9, C).

Selection of normal-shaped tubers from a Green Mountain and an Irish Cobbler lot from 1917 to 1922 did not produce stock free from spindling tuber. In fact, the Irish Cobbler lot showed over 90 per cent spindling tuber in 1922, or an increase of about 90 per cent in six seasons. Although the conditions for field infection in this lot, being grown near diseased stock in experimental plots, were very favorable, nevertheless this indicates that tuber selection alone does not insure freedom from this malady.

With the spindling-tuber disease then, as with other insect-borne diseases of the potato, the futility of attempting control by means of tuber selection alone is very evident from the results of insect transmission.

HILL SELECTION

In 1918 hills were selected as healthy, both next to mosaic hills and also with varying numbers of healthy hills between them and the nearest mosaic hill in the same row, and those next to diseased hills produced more mosaic progeny than the others (*40, p. 334*). In 1919 Green Mountain hills that apparently were healthy were dug in two places at progressively later dates in the season. One set was secured at the Presque Isle laboratory plots and consisted of 50 hills each grown in

the row next to a mosaic hill. The other was obtained at Aroostook Farm in a rogued plot about 15 meters from the nearest unrogued mosaic hills. In each place 10 hills were dug on each of five dates—August 2, 11, 20, 30, and September 9. The progeny of the five groups of hills were mosaic respectively in 67, 68, 70, 89, and 93 per cent in the laboratory stock and in 0, 7, 0, 2, and 26 per cent in the farm stock. Therefore, in the laboratory hills, next to mosaic ones, there was a high percentage of mosaic infection by August 2 and probably only chance escaping of inoculation prevented all hills from becoming diseased by August 30. In the farm plot, 15 meters from mosaic hills, there was little infection until September 9, and then much less than at the laboratory. The two sets of hills were not only of the same variety but to a large extent of the same strain. Evidently, proximity to mosaic hills greatly increased infection, apparently through easier dispersal by virulent insects. The small amount of infection that occurred at the farm and that was manifested chiefly in the last harvest probably was due to virulent insects, soil and other factors appearing to be negligible as possible causes.

An experiment parallel to the preceding consisted in removing one tuber from every one of 30 hills in each of the two places on each date except the last, when every hill was dug usually with more than one tuber. The results were the same except that the percentage of infection generally was higher. Another parallel experiment consisted in selecting apparently healthy hills next to mosaic ones in a field, at the farm, that contained Green Mountain plants with 80 per cent mosaic. One hundred pounds of tubers were dug on August 7, and a like quantity again on September 8. The progeny of the two lots were, respectively, 46 and 84 per cent mosaic. These results are similar to those of the series of harvests made at the laboratory.

In 1920 conditions of uncontrolled field transmission were presumably varied by the selection of hills at different times and at different places on the same farm. The data are presented in Table XXVII.

In field A, proximity to mosaic hills increased the amount of infection and proximity to leaf-roll hills was necessary for infection. The sudden increase of infection from the August 12 harvest to that of August 23 is to be explained as follows: In this field certain recommended methods of spraying for aphid control were being tested and meanwhile the approximate number of aphids was determined and recorded frequently. About 95 per cent of these insects were potato aphids (*Macrosiphum solanifolii* Ashm.). These were present by July 17, increased most rapidly during the second week in August until there were over 2,000 to a plant on the average, and decreased rapidly and disappeared during the third week in August mostly because of a fungus disease that followed the inception of a period of cloudy, humid weather. The aphids of the other species (determined as *Aphis sp.* by Dr. Edith M. Patch), were extremely localized on occasional plants, being apparently slow in dispersing. Presumably, the greatest dispersal of aphids occurred about August 13, when they were most numerous and when the second harvest of tubers was completed. Apparently the high level of tuber infection was reached by August 23, in the healthy hills adjacent to diseased ones, indicating a 10-day period to be necessary for diffusion of the virus to the tubers if the chief cause of infection were the potato aphids. This is the time for such diffusion as shown on page 97. The

fact that leaf roll did not reach the tubers in hills adjacent to leaf-roll hills in this field until after August 13, and then only in small amount, may be due to the greater difficulty of effective leaf-roll inoculation compared with mosaic, or to slower diffusion of the virus in the plant, or to both.

TABLE XXVII.—*Mosaic and leaf roll uncontrolled inoculations in the field in 1920*[1]

Location in 1920.[2]	Series.[3]	Hills.	Progeny, 1921. Tuber units.	Mosaic.	Leaf roll.
				Per cent.	*Per cent.*
In field A, next to mosaic hills	1-A	10	39	3	0
	1-B	10	42	5	0
	1-C	10	42	69	0
	1-D	10	44	48	0
In field A, next to leaf-roll hills	2-A	10	41	2	0
	2-B	10	43	9	0
	2-C	10	38	16	0
	2-D	10	38	18	18
In field A, in healthy plot	3-A	10	41	0	0
	3-B	10	48	6	0
	3-C	10	42	0	0
	3-D	10	39	15	0
In field B (rogued), next to sites of rogued hills	4-A	10	42	0	0
	4-B	10	40	13	0
	4-C	10	45	7	0
	4-D	10	31	19	0
In field B, not next to sites of rogued hills	5-A	10	42	0	0
	5-B	10	44	2	0
	5-C	10	44	5	0
	5-D	10	37	0	0
In field C, next to mosaic hills	6-A	10	48	8	0
	6-B	10	47	17	0
	6-C	10	47	15	0
In field C, not next to diseased hills	7-A	10	44	5	0
	7-B	10	47	13	0
	7-C	10	45	16	0
Irish Cobblers in field C, next to leaf-roll hills	8-A	5	25	16	0
	8-B	5	24	42	0
	8-C	5	19	5	0
	8-D	5	25	20	0
Irish Cobblers in field C, not next to leaf-roll hills	9-A	5	25	4	0
	9-B	5	22	18	0
	9-C	5	23	0	0
	9-D	5	25	4	0
In field D (rogued), on soil in which mosaic plants grew in 1919.[4]	10-A	10	49	2	0
	10-B	10	48	0	0
	10-C	10	47	6	0
	10-D	10	43	5	0
In field D, on soil in which healthy plants grew in 1919	11-A	10	41	0	0
	11-B	10	47	2	0
	11-C	10	47	2	0
	11-D	10	44	7	0

[1] Green Mountains except for Series 8 and 9.
[2] Except for field D, with 2 years elapsing with no potatoes grown on the soil.
[3] Each series consists of subseries according to the dates of harvesting, which are, respectively, July 29–30 (A), Aug. 12–13 (B), Aug. 23–24 (C), and Sept. 15 (D).
[4] No volunteers present because all tubers in the soil were killed by a severe winter with snowfall light in the early part.

In fields B and D, which were rogued of diseased hills as soon as the symptoms were observed, the small amount of infection found by August 12 may be the result of insect transmission occurring before the roguing was performed. A comparison of Series 3, 10, and 11 shows that as much mosaic may be contracted by plants in a healthy plot in the same field with mosaic plants and plots as by plants grown on soil that supported a mosaic plot the year before (*40, p. 335–36*). A comparison of Series 1 and 6 shows that considerable mosaic infection reached the tubers of hills next to diseased hills by August 13 in one field, but not in the other, and a comparison of Series 2 and 8 shows that it was only in the former field that leaf-roll infection reached the tubers of hills next to diseased hills by September 15. The former field was about three times as far as the latter from the nearest known winter host of the potato aphids (*36*) and possibly became infested later or to a less extent, or both.

In 1921 hill selections were made in two Irish Cobbler fields containing, respectively, 5 and 15 per cent of hills producing spindling tubers, and in a Green Mountain field containing 1 per cent. The results, given in Table III, indicate that hill selection was useless for eliminating disease, though it reduced the increase otherwise occurring without hill selection. It is not surprising that hill selection in a field containing disease is often disappointing. Murphy (*29, p. 45–47*) and Quanjer (*39, p. 142*) have had the same experience. When as low as 5 per cent of diseased hills may contaminate the majority of the healthy hills in the same field, the hill-selection method clearly has limitations.

REMOVAL OF DISEASED HILLS

The removal of diseased hills, or roguing, is in the broad sense a method of hill selection. It differs from hill selection in the strict sense chiefly by using healthy hills after removing the diseased hills that may serve as sources of infection, whereas the latter uses healthy hills that have been more or less exposed to infection throughout the season. The effectiveness of roguing is determined by several factors. Correct diagnosis, thoroughness at each inspection, complete removal of each rogued hill from the field, and several inspections at the proper times are necessary.

Roguing with no insect spraying and with no great degree of isolation has been tested for several years, 1917–20, by the writers (*45, p. 270; 40, p. 332; 41, p. 77*). With variations according to the season and degree of proximity to diseased plots, the general results have been to keep the amount of mosaic in the stock about the same from year to year, between 13 and 30 per cent, except that roguing in 1920 was followed in 1921 by 35 per cent and 65 per cent in Green Mountains and Bliss Triumphs. This was a gain over conditions in unrogued parts of the same strains. Why the removal presumably of all diseased hills has not eliminated the disease, or at least steadily decreased the percentage, is not certain. Possible causes are masking of symptoms at the time of roguing, dispersal of transmitting insects from rogued plants before or after these plants are pulled and insect dispersal from unrogued plots.

Roguing accompanied by isolation from unrogued diseased stock and by insect spraying will be considered later.

TABLE XXVIII.—*Effect of proximity on healthy and diseased potatoes*

Field.	Sample.	History of good stock. 1921 Variety.	1921 Location of sample in field.	1921 Disease.	1921 Aphids.	1922 Mosaic.	History of near-by potato fields. 1921 Variety.	1921 Mosaic.	1921 Distance from good stock.	1921 Direction from good stock.
				Per cent.		*Per cent.*		*Per cent.*	*Meters.*	
31	1	Green Mountain	Northeast	Less than 1 mosaic		0				
	2	do	East	do		0				
	3	do	Southeast	do		8				
	4	do	North	do		26		10–15	70–170	North, east, and northeast.
	5	do	Center	do		3	Green Mountain			
	6	do	South	do		0		(1)	100	Northwest.
	7	do	Northwest	do		0				
	8	do	West	do		0				
	9	do	Southwest	do		0				
34	1	do	Northeast	do		0				
	2	do	East	do		0				
	3	do	Southeast	do		0				
	4	do	North	do		0	Irish Cobbler	1+	(2)	On west and south.
	5	do	Center	do		0	Rose 4	10	(2)	North.
	6	do	South	do		8	Green Mountain	98	70	Do.
	7	do	Northwest	do		0				
	8	do	West	do		0				
	9	do	Southwest	do		19				
37	1	do	North	4 mosaic	8 on 5 leaves.	0				
	2	do	North center	do	do	8	Green Mountain	38	60	South.
	3	do	Center	do	do	16	Irish Cobbler	(3)	(2)	East. West.
	4	do	South center	do	do	0				
	5	do	South	do	do	7				
38	1	do	Northwest	4 in American Giant volunteer plants; none in Green Mountain.	Abundant	7				
	2	do	West	do	do	32				
	3	do	Southwest	do	do	58				
	4	do	North	do	do	56	Bliss Triumph	40	(4)	All sides of field.
	5	do	Center	do	do	14				
	6	do	South	do	do	9				
	7	do	Northeast	do	do	1				
	8	do	East	do	do	46				
	9	do	Southeast	do	do	17				

[1] High. [2] Adjacent. [3] Apparently healthy. [4] Adjacent; healthy field only 12 meters wide.

TABLE XXVIII.—*Effect of proximity on healthy and diseased potatoes*—Continued

Field.	Sample.	History of good stock. 1921 Variety.	1921 Location of sample in field.	1921 Disease.	1921 Aphids.	1922 Mosaic.	History of near-by potato fields. 1921 Variety.	1921 Mosaic.	1921 Distance from good stock.	1921 Direction from good stock.
				Per cent.		*Per cent.*		*Per cent.*	*Meters.*	
42	1	Green Mountain	Northeast corner	Less than 1	13 on 5 leaves.	1	Rose 4	([5])	([6])	Northwest corner.
	2	do	Southeast corner	do	do	1	Irish Cobbler	([5])	([7])	East of southeast corner.
	3	do	Middle	do	do	13				
	4	do	Northwest corner	do	do	4	do	([5])	([2])	Southwest.
	5	do	Southwest corner	do	do	66	Green Mountain	66	100	Southwest corner.
43	1	do	Northeast corner	Trace in field	3 on 5 leaves.	9	do	2	20	Northeast.
	2	do	Middle	do	do	0	Irish Gem	12		
	3	do	Southwest	do	do	0				

[5] Apparently none. [6] Across road. [7] Across railroad. [2] Adjacent.

SELECTION AND ISOLATION OF HEALTHY STOCKS

It is possible to find fields that are healthy, either absolutely or comparatively. Then it becomes important to know what to expect of such fields under known conditions of isolation from diseased stocks, and what to do with stock from such fields to maintain the existing state of health. Usually when healthy stocks are secured by potato growers they are planted in such a way in relation to diseased stocks that there is a chance to study the effects of proximity rather than of isolation, so that proximity will be considered here also.

The results of planting rows of healthy stock alternating with mosaic stock have been described on page 102. In 1918 and 1919 rows of Irish Cobbler plants contracted more leaf roll next to leaf-roll rows than when farther removed (*41, p. 76–77*). As in the mosaic experiments, the effects of the various factors, such as contact, soil, and virulent insects, can not be distinguished. Murphy has disclosed the spreading of mosaic and leaf roll across several rows in various parts of Canada (*29, p. 47–51, 64–65*). Krantz and Bisby state that mosaic dwarf spreads rapidly in Minnesota (*21, p. 19–22*).

In 1920, two Green Mountain fields with less than 1 per cent mosaic were observed. One was small, consisting of four rows, and was planted about 30 meters distant from four rows of diseased Irish Cobblers that came from a farm where all degeneration diseases are present. In 1921, about 10 per cent of the hills were diseased, with leaf roll, mild mosaic, rugose mosaic, leaf-rolling mosaic, and spindling-tuber disease. Some disease appeared even in volunteers from tubers left in the field before these were destroyed by chewing insects. The other field was large, and was planted about 50 meters distant from an American Giant field with 24 per cent of the hills mosaic. Samples and bulk stock from this field contained less than 1 per cent again in 1921.

Two growers in southern Maine isolated very healthy Green Mountains sufficiently in 1921 for the stock in 1922 (seen by the writers) to have less than 1 per cent disease, one grower giving the distance of isolation as about one-fourth mile.

In 1921, the writers selected samples in several fields planted with healthy stock and located at different distances from fields planted with diseased stock. During the growing season observations on the percentage of diseased plants as well as on the aphid infestation were made. Peck samples were harvested from different parts of each field. These were planted in 1922 and readings on the percentage of mosaic were taken. Further data on these observations are presented in Table XXVIII.

The results in Table XXVIII disclose that the mosaic percentage increased in each of the stocks and that the greatest increase obtained in field 38, where no hill was farther than 6 meters from Bliss Triumphs having 40 per cent mosaic and where there was a heavy aphid infestation. The stock in field 42 happened to be the one described previously as being 50 meters distant from mosaic American Giants in 1920 and as showing less than 1 per cent mosaic in 1921. Apparently, the location and other conditions of this stock were more favorable for infection with mosaic in 1921 than they had been in 1920, for one sample gave progeny with 66 per cent, and the bulk stock of this field showed 15 per cent mosaic in 1922. The results in this table show that mosaic sometimes spreads very readily from diseased to adjacent healthy lots, that prox-

imity and heavy aphid infestation together increased the spread of mosaic, and that with greater isolation than obtained in these fields it may be possible to maintain stock mosaic free if once the disease is eliminated.

Isolation of a half mile in England has prevented infection while proximity increased it (*10*, *30*).

INSECT CONTROL

Aphids have proved to be effective transmitting agents in a number of the experiments described in this paper. Although it may not be possible to control aphids sufficiently to prevent transmission in the field in all times and places, it at least is necessary unless there is no disease present. There are two distinct phases of the problem of insect control in relation to disease transmission. The presence of transmitting insects in a partly diseased field is to be expected to result in late-season infection of healthy hills that can not be distinguished and that therefore can not be eliminated in the current season. In a healthy field the mere presence of insects is harmless in relation to disease, but the dispersal of transmitting insects to either kind of field from a wholly diseased field is undesirable. In some situations it may be possible to prevent interhill transmission before removing the diseased hills and yet be impossible to prevent new contamination from other fields, while in other situations it may be possible to prevent interfield transmission and yet be impossible to avoid an infestation that causes interhill transmission.

In endeavoring to follow the recommendation of entomologists regarding aphid control, the writers have formed the opinion that spray methods reported to be effective in more southern regions, at least in preventing directly injurious infestations, are not so useful in preventing transmission in northeastern Maine. In this region high-ridge culture is practiced, so that many leaves lie on the surface of the soil and support aphids that can not be touched by the spray and that are a permanent source of infestation. Also, the tops in healthy fields grow so large that they form an expanse of foliage through which it is often impossible to walk without stepping over plants and in which it is difficult to drag undershot nozzles and to use them effectively for covering the leaves. Expensive applications of nicotine solution in 1921 reduced aphids in two four-row sections in a seed plot containing 19 such sections. The seed plot was rogued of about 10 per cent of the diseased hills, partly after aphids were present, and these two sections had progeny with about 9 per cent diseased as compared with from 15 to 18 per cent for the rest of the seed plot. The same methods used again in 1922 were effective in killing all aphids except on the leaves next to the soil. The seed plot of 1922 was a half mile further from rose bushes than the seed plot of 1921, and the potato aphids arrived about two weeks later than at the site of the 1921 plot, following roguing of all mosaic hills. The progeny in 1923 will indicate the value of isolation of potatoes from rose bushes (*36*) in reducing the spread of mosaic and the value of the spraying in reducing the spread of the spindling-tuber disease. The latter could be rogued only in part of the seed plot planted by tuber units.

In 1918, several tubers from healthy Bliss Triumph hills grown in cages were selected to begin a strain grown in insect-proof cages in the following seasons of 1919 to 1922. Every tuber from this caged stock has produced healthy plants. These cages were located in the field near potatoes with high percentages of mosaic and leaf roll, and during the same period

uncaged Bliss Triumph stock of the same original strain as the caged plants grown in the same locality increased from 15 to 84 per cent mosaic. Similar observations were made by Krantz and Bisby (*21, p. 18–19*). That the protective effect of caging is due to insect exclusion rather than to its modification of climatic factors is shown by the many cases described previously of infection within cages by introducing virulent insects.

In this connection it is interesting to note that Murphy (*31*) finds that capsid bugs and jassids transmit leaf roll of potato in Ireland, where those insects apparently are more important in this respect than aphids. Elmer (*13*) reports successful cross inoculations by transferring mealy bugs (*Dactylopus* sp.) from mosaic infected Solanum and cucurbit plants to healthy cowpea (*Vigna catjang*) seedlings. Such observations confirm the general assumption that possibly a number of different insects may be found which transmit these diseases.

GENERAL CONCLUSIONS

In addition to a subsequent summary including the various facts demonstrated in this paper, two general conclusions may be pointed out, relating, respectively, to the rôle of diseases in the degeneration of potatoes and to the general influence of region on the degeneration-disease problem.

"Running-out" is generally recognized as relating to plants which for various reasons fail in the maximum production. Unfavorable soil and weather conditions, senility, reversion, and loss of vigor due to prolonged asexual reproduction are some of the causes to which degeneration in plants has been ascribed. While such factors as favorable soil and climate undoubtedly play a very effective rôle in the proper development of plants, it must also be recognized from the comparatively recent investigations on degeneration diseases in plants that no matter how favorable the conditions for development, a diseased plant will be less productive than a healthy one of the same species and variety in the same environment.

Perhaps in no other plant has the theory of senescence been so frequently mentioned as with the so-called degeneration or deterioration of the potato. Abnormalities due to mosaic, leaf roll, and spindling tuber heretofore have frequently been ascribed to the asexual reproduction of the potato. In view of the recent findings regarding the nature and infectiousness of these maladies, it is no longer necessary to mention senility in connection with them, as pointed out by Cotton (*10, p. 163–164*). When one realizes that a large percentage of healthy plants exists in many of the potato varieties propagated asexually for many years, one questions rather seriously whether senescence plays any rôle whatsoever. In this connection, as Cotton (*10, p. 164*) has pointed out, many of our most persistent weeds continue to reproduce very successfully almost entirely through asexual reproduction.

The evidence on the presence and importance of degeneration diseases of the potato also shows that a knowledge of the pathological significance of an abnormal condition of a plant is as necessary as a knowledge of its cultural or genetic relations, if reliable deductions are to be drawn (*10, p. 164*).

Furthermore, it is apparent that these degeneration diseases must be recognized in any authentic comparison of strains of potatoes in the

same variety. It is possible that differences in performance between strains of the same variety result from varying percentages of those degeneration diseases and not merely from other variations, such as vigor, as is frequently designated. Disease-free strains of the same variety should be grown under absolutely uniform conditions for several seasons in order to permit accurate testing of the supposed inherent differences between strains of a variety.

Regarding the general effect of the region the various experiments detailed in this paper tend to show that the potato degeneration problem is similar in the northeastern United States to the same problem in other parts of America (including Canada) and in parts of Europe (including the British Isles). It is a complex problem, involving several diseases that react differently to transmission agents, varieties, and other environmental factors. While this general similarity is true, it is also true that the problem because of its complexity may vary greatly from one locality to another. It follows then that control measures must be worked out for different sets of conditions, following research based initially on the general principles now fairly well understood.

Two examples may be given of striking differences between regional problems. These may or may not be referable fundamentally to climate, bearing in mind that the preference given to varieties in a given region or the prevalence of insects transmitting a disease may be determined by climate. The presence of the spindling-tuber disease in at least five widely separated States of this country, with its apparent absence from Europe, is a difference that, regardless of its cause, complicates the situation because of the elusiveness of vine symptoms. The presence of net necrosis as a tuber symptom of leaf roll in at least two States of this country and in Japan also is a feature that has not been noted in Europe. A third difference, consisting of the successful development and use of a leaf-mutilation method of inoculation in at least three parts of this country may disappear when the method is tried in other countries (p. 54). In view of such differences and the differences reported for parts of the same country, the effectiveness of certain control measures in one region can not be trusted as a reliable indication of their usefulness in another region.

With the degeneration problem showing somewhat different aspects in one region from another, with seed being transported from northern to southern regions to reduce injury from degeneration, and with the need for the use of all scientific results, it is desirable that it at least be possible to identify a given degeneration disease in different places. This is not yet possible because of the modifying effects of climate and variety upon the symptoms, and because of the variation of climate and varietal preference from one region to another. Growing parts of the same tuber, with many tubers, and in different regions, is helpful but not always practicable. Undivided tubers are not reliable for comparison unless they are produced under conditions where no infection of the parent vines is possible. Great usefulness is in store for methods that will readily identify the yet unknown causal agencies, whether organisms or compounds, of the several diseases, somewhat as phloëm-necrosis microanalysis has been used by Quanjer (*39, p. 132–37*) to detect an apparently consistent symptom of leaf roll. Furthermore, it is quite apparent that the identification of the causal agencies, whether by analysis or by culture, will ultimately result in an authentic classification of the so-called virus diseases.

SUMMARY

(1) Degeneration diseases of potatoes, in the absence of known identifying causes, are symptom complexes whose elementary unit symptoms can and should be determined in the same standard variety or varieties and in the same environment.

(2) Research with these diseases is developing a somewhat distinct technic and terminology.

(3) In the Green Mountain variety, several degeneration diseases have been distinguished and transmitted—namely, mild mosaic, leaf-rolling mosaic, rugose mosaic, streak, leaf roll, spindling-tuber disease, and unmottled curly dwarf.

(4) In Green Mountains, mild mosaic was not transmitted by contact except in stem and tuber grafts.

(5) A leaf-mutilation method of inoculation has certain advantages over other methods. In Green Mountains this method transmitted mild mosaic, with the effectiveness increased by insect-cage or greenhouse conditions as compared to open-field conditions, by inclosure within a damp chamber, and by repetition.

(6) In Green Mountains aphids (*Macrosiphum solanifolii* Ashmead) transmitted mild mosaic, both alone and in combination with leaf roll and with spindling tuber, while negative results were secured with flea beetles (*Epitrix cucumeris* Harris) and Colorado Potato beetles (*Leptinotarsa decemlineata* Say.).

(7) In Green Mountains, aphids from plants with a "curly-dwarf" combination apparently consisting of leaf-rolling mosaic and spindling tuber together, transmitted the curly-dwarf combination to part of a hill and spindling tuber alone to the other part, distinction being made between different tuber units of the second generation.

(8) Leaf-mutilation inoculation transmitted both rugose mosaic and streak readily in Green Mountains.

(9) Leaf roll was transmitted neither by contact except in grafts, nor by leaf-mutilation inoculation.

(10) Spindling-tuber disease is characterized, and proofs given of its being a degeneration disease, spreading in the field, being perpetuated by the tubers (shown in part by mechanical measurements), and being transmitted by tuber grafts, stem grafts, leaf mutilation, and aphids.

(11) Unmottled curly dwarf was transmitted by leaf-mutilation inoculation and by aphids.

(12) Combinations of symptoms exist that include more than one degeneration disease in the same plant. Aphids sometimes transmit only one disease from such a plant, but more often transmit the combination.

(13) In Irish Cobblers, leaf roll was transmitted by grafting and by aphids but not by leaf-mutilation inoculation, which is successful with all other degeneration diseases tested.

(14) In New White Hebrons in 1921, leaf-roll and net-necrosis percentages increased with the average weight of the tubers.

(15) Leaf-mutilation inoculation of Green Mountains in 1920 effected intervarietal transmission of rugose mosaic, a combination of leaf-rolling mosaic and spindling tuber, and unmottled curly dwarf, but was ineffective with mild mosaic and leaf roll. Comparison inoculations with aphids with four of the sources of inoculum gave similar results, transmitting rugose mosaic to Green Mountains from curly-dwarf plants in the Rural group.

(16) Leaf-mutilation inoculation of Green Mountains in 1921 effected intervarietal transmission of streak, rugose mosaic and streak together, rugose mosaic, leaf-rolling mosaic, and mild mosaic in insect cages (uncontrolled in the open field), but not leaf roll. With spindling tuber (uncontrolled in the open field) present in the progeny, leaf-rolling mosaic having been transmitted formed a curly-dwarf combination. Rugose mosaic was thus transmitted from Carman No. 3 (Rural group) to Rural New Yorker, and from this and two seedling varieties to both Green Mountains and Irish Cobblers, but the symptom complexes were not identical in any two varieties.

(17) Streak did not appear the same before and after tuber perpetuation.

(18) Comparison inoculations with juice in capillary glass tubes were much less effective than leaf mutilation, with rugose mosaic and streak.

(19) Aphid inoculations of Green Mountains in 1921 effected intervarietal transmission of leaf-rolling mosaic from curly-dwarf plants in three varieties of the Rural group, of unmottled curly dwarf and spindling tuber (separately) from Irish Cobblers, of rugose mosaic from one seedling variety, and of leaf-rolling mosaic and rugose mosaic together from two seedling varieties. Aphids also transmitted rugose mosaic to Irish Cobblers from two seedling varieties.

(20) Leaf-mutilation inoculations of Green Mountains in 1922 resulted in current-season symptoms of streak, rugose mosaic and streak combined, and rugose mosaic with and without streaking. Comparison inoculations with mild mosaic, leaf roll, and spindling tuber gave no current-season symptoms in the open field. Comparison inoculations under insect cages gave current-season symptoms with mild mosaic and spindling tuber. Several different symptom complexes yielded only rugose mosaic as the current-season effect of inoculation, sometimes with rugose mosaic somewhat masked in the original complex. Results with Green Mountains were somewhat duplicated in Irish Cobblers and Bliss Triumphs, but some varietal modification of symptoms apparently occurred in these and seedling varieties.

(21) In greenhouse inoculations, transmission of mild mosaic from Bliss Triumphs to Green Mountains was effected to some extent with juice in capillary glass tubes, but not with immersion of a split stem in diseased juice. It was effected more readily with leaf mutilation as the number of inoculated leaves was increased, and more readily with aphids as the number of individual insects was increased, being possible with one individual aphid.

(22) In greenhouse leaf-mutilation inoculations, rugose mosaic was transmitted from a seedling variety to Green Mountains and from Green Mountains to Irish Cobblers, but leaf roll was not transmitted from Irish Cobblers to Green Mountains.

(23) Interspecific inoculations with leaf mutilation and aphids indicate that tobacco mosaic is not identical with potato mild mosaic, that tomato is susceptible to both of these mosaics and also to potato rugose mosaic, and that nightshade (*Solanum nigrum* L.) is susceptible to potato mild mosaic. Raspberry mosaic seems harmless to potatoes.

(24) The various degeneration diseases of potatoes are different as to their economic importance resulting from their distribution and effect upon yield rate and quality.

(25) Natural transmission by insects contributes to the difficulty of the control problem.

(26) Perpetuation occurs in tubers and not in soil alone.

(27) About 10 days were required for the mild mosaic virus to diffuse from inoculated leaves to the tubers.

(28) Mosaic plants from the same seed tubers sometimes show different symptom complexes in different environments. Mottling is suppressed by southern regions and by higher temperatures. Dwarfing of the tubers, and therefore reduction of yield rate, was more pronounced in a southern region.

(29) Shading tended to increase mosaic mottling and decrease leaf rolling.

(30) In duplicate plots leaf roll and mosaic were contracted by healthy lots growing between rows of diseased lots, more in some regions than others.

(31) Selection of tubers without knowledge of the parent plants can not eliminate seed from diseased plants infected late the preceding season.

(32) The digging of selected healthy hills progressively later in the growing season was correlated with greater numbers of aphids and with greater amounts of disease in the progeny.

(33) Hill selection in fields containing diseased plants throughout the growing season is disappointing as a means of eliminating disease, but sometimes gives better results than using unselected stock from the same field.

(34) Proximity and a heavy aphid infestation increased the spread of mild mosaic, while sufficient isolation from diseased stocks reduced it so that a state of freedom from the disease was maintained. Isolation by 30 meters was insufficient, and over 400 meters was sufficient.

(35) Conditions that reduced aphid dispersal from diseased to healthy hills also reduced the amount of disease transmission.

(36) Potato degeneration is largely, and possibly is entirely, a result of the increase of, and injury by, certain degeneration diseases.

(37) The potato degeneration-disease problem is on the whole similar for all potato-growing regions, but is complex enough to vary somewhat from one region to another.

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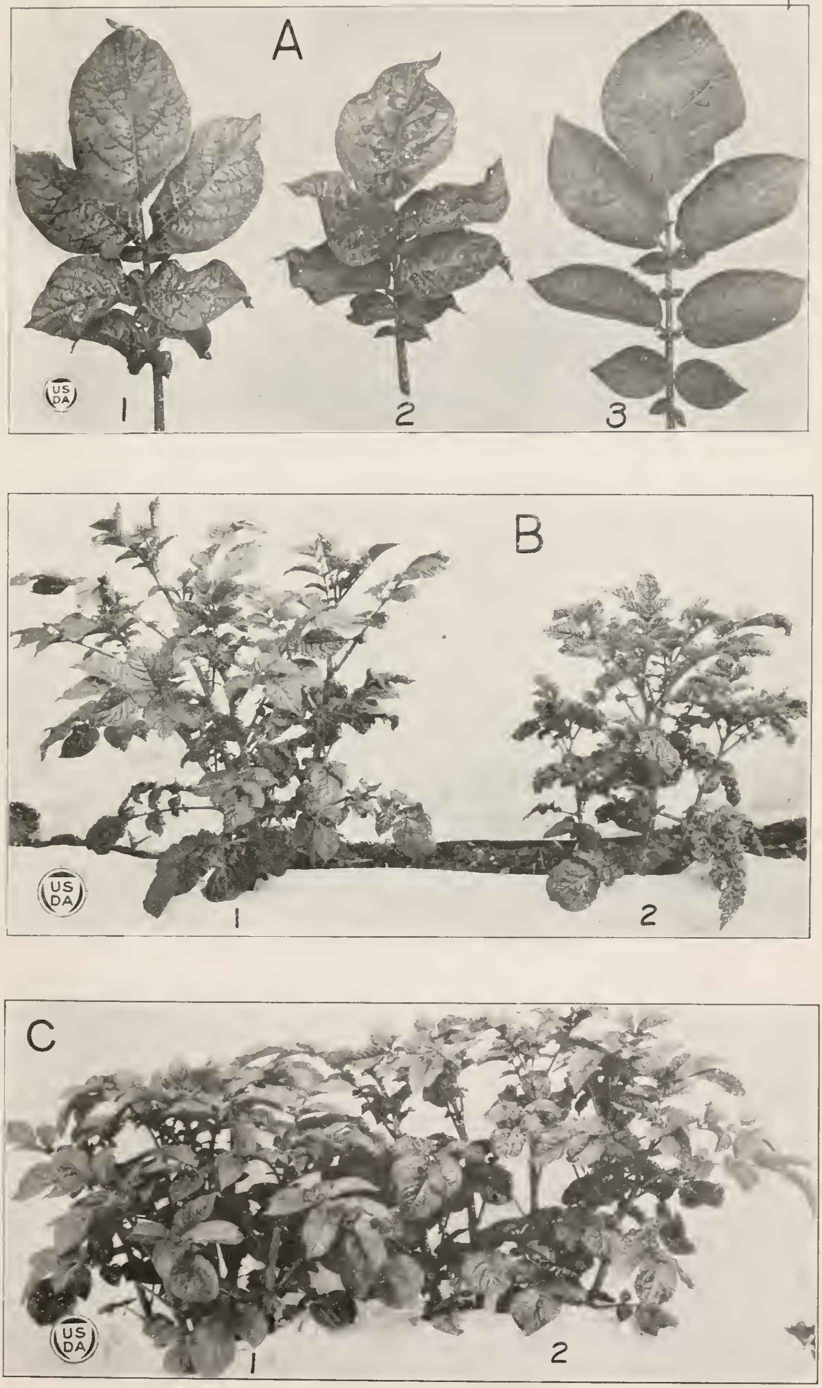
PLATE 1

A.—(1, 2) Leaves from mosaic Green Mountain plant. (3) Leaf from healt
Green Mountain plant.

B.—(1) Mild mosaic Green Mountain. (2) Medium plus mosaic Green Mounta

C.—(1) Healthy plant from half-tuber seed piece. (2) Mild mosaic plant from a ha
tuber seed piece. Both from same hill of the preceding season. Green Mount
variety.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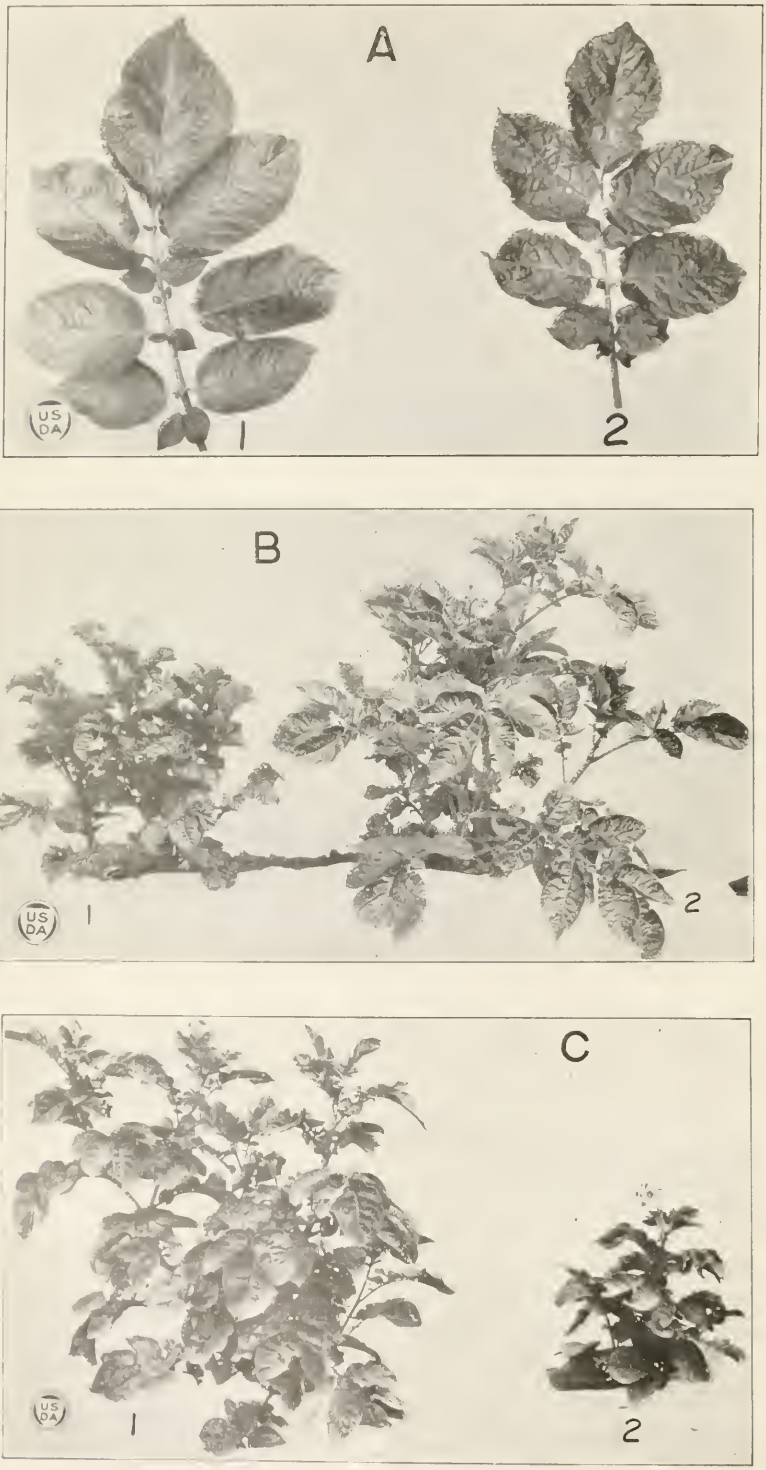
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118[2]

PLATE 2

A.—(1) Healthy leaf from vine from same tuber as inoculated plant also grown in cage. (2) Mild mosaic leaf from Green Mountain plant inoculated with inoculum from mild mosaic Green Mountain in cage.

B.—(1) Plant with leaf-rolling mosaic and spindling tuber, forming a curly-dwarf combination. (2) Leaf-rolling mosaic plant. Green Mountain variety.

C.—(1) Leaf-rolling mosaic Green Mountain plant diseased as result of aphid inoculation in preceding season from parent of plant in C, 2. (2) Curly-dwarf plant of Uncle Sam variety (Rural group).

PLATE 3

A.—(1 and 2) Curly-dwarf Green Mountain. Source of inoculum for inocula
with aphids in cages in 1921. (Inoculated Green Mountain in B.)

B.—(1) Spindling-tuber vine from same 1921 hill as curly-dwarf plant, but inf
with only one disease from the combination. (2) Curly-dwarf or spindling-tuber
rolling mosaic plant. Second generation to aphid inoculation in cages (sour
inoculum in A). Variety Green Mountain.

C.—Rugose mosaic and healthy Irish Cobbler vines. Mosaic plant is progeny
vine apparently showing streak the previous season.

118


A
1
2
3


B


C

118[4]

PLATE 4

A.—(1) Rugose mosaic Green Mountain produced by aphids dispersing from rugose seedling in cages. (2) Healthy Green Mountain from same 1921 hill as preceding infected plant. (3) Rugose mosaic seedling. Source of juice for aphid inoculation in cages.

B.—Rugose mosaic and streaking in top of plant. Variety Green Mountain.

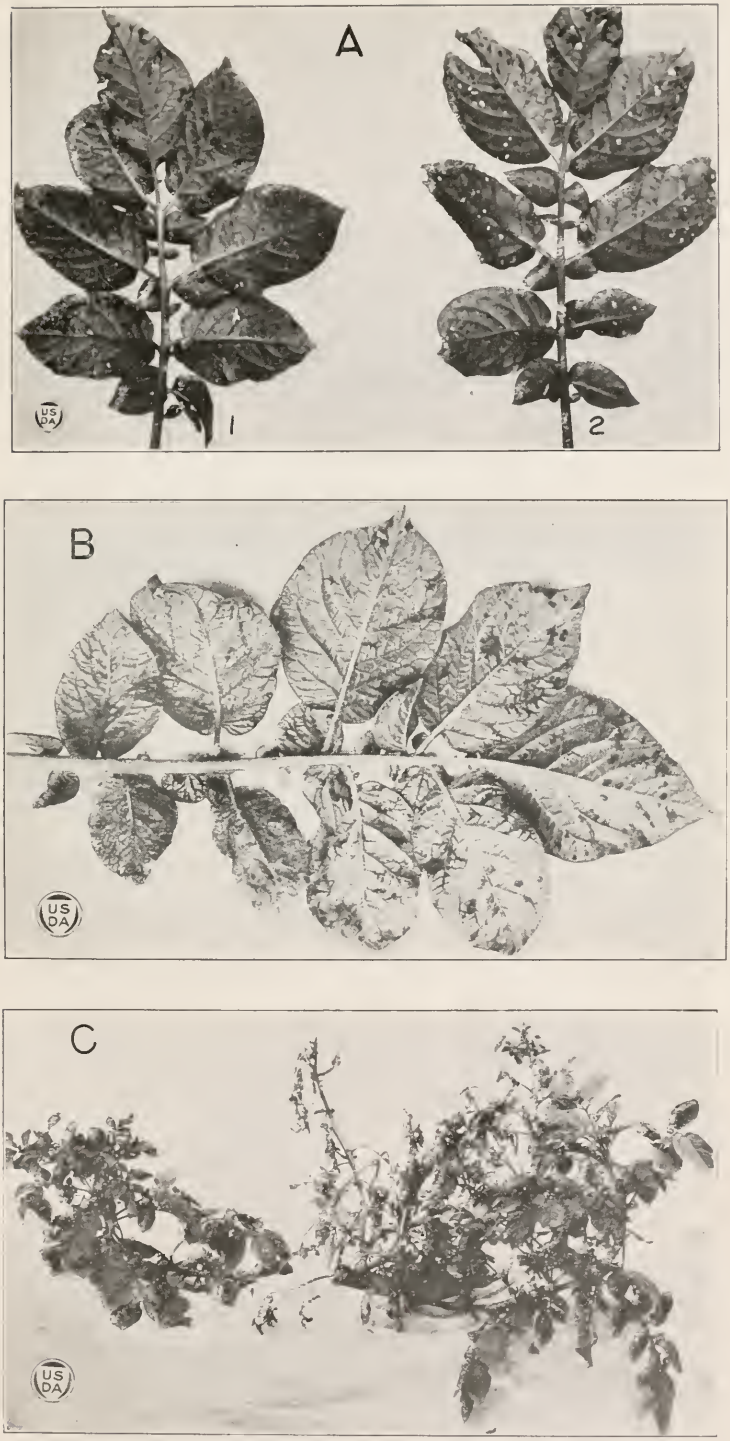
C.—Green Mountain plant healthy below, leaf dropping in middle, rugose mosaic above.

PLATE 5

A.—Leaves from Green Mountain plant inoculated with juice from streak seedlin showing spotting, streaking, and burning. Some dead spots apparently spreadi along veins producing a streaked appearance.

B.—Leaf of seedling with streaking, spotting, and burning of streak.

C.—Green Mountain plant showing streak resulting from leaf mutilation inoculati with juice from streak seedling when plant was about 15 cm. high. July, 1921.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2

118[6]

PLATE 6

A.—(1) Early stage of streak on seedling. (2) Mosaic dwarf on seedling.

B.—(1) Streak plant in second year of disease, progeny of plant in same series as that in Plate 5, C. (2) Healthy plant of control lot. Green Mountain variety.

C.—(1) Healthy hill. (2) Leaf-roll hill. These hills were in the same tuber unit.

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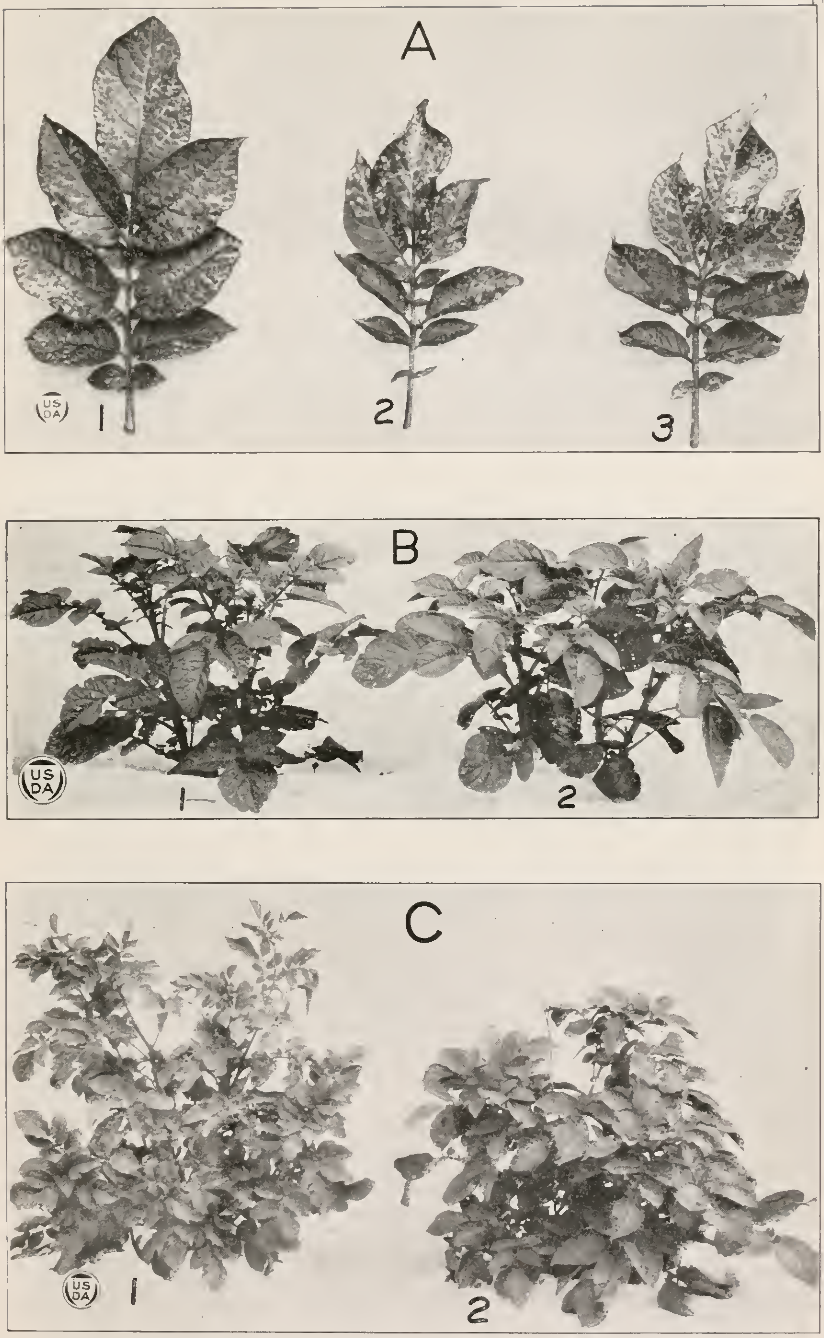
PLATE 7

A.—(1) Top leaf from healthy Green Mountain. (2, 3) Top leaves from spindlin tuber Green Mountain. From plants of C (1, 2).

B.—(1, 2) Same hills as shown in C (1, 2), earlier in the season (July 6).

C.—(1) Spindling-tuber Green Mountain from half-tuber seed piece. (2) Health Green Mountain from quarter-tuber seed piece. Same hills as shown in B, but lat in the season (August 29).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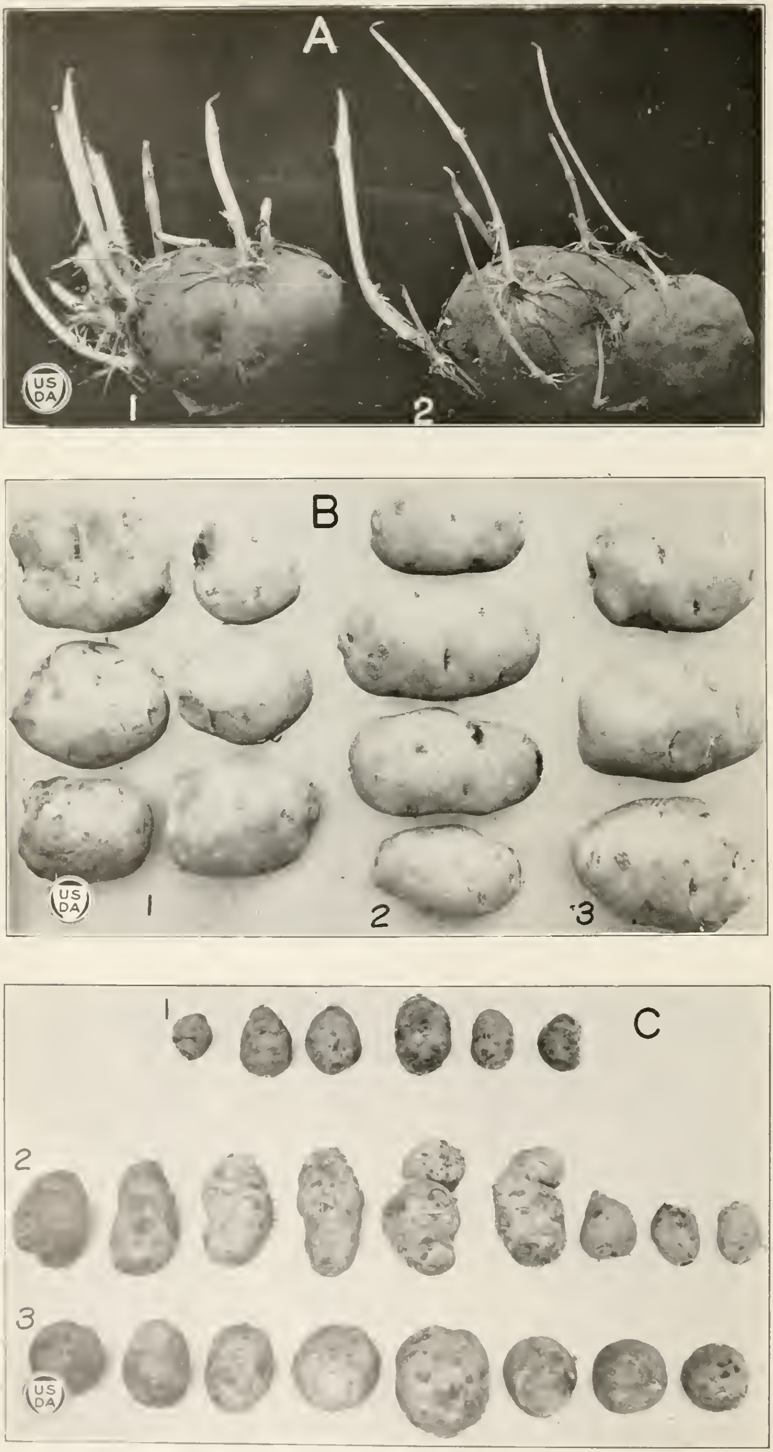
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118 8

PLATE 8

A.—(1) Irish Cobbler tuber, normal shape, side view, after sprouting. (2) Irish Cobbler tuber, spindling-tuber shape, from same lot of seed.

B.—(1) Healthy Green Mountain, control to B, 2. (2) Spindling tuber in second generation as a result of growing in cage with aphids and spindling-tuber Irish Cobbler in first generation in 1921. (3) Spindling-tuber Irish Cobbler.

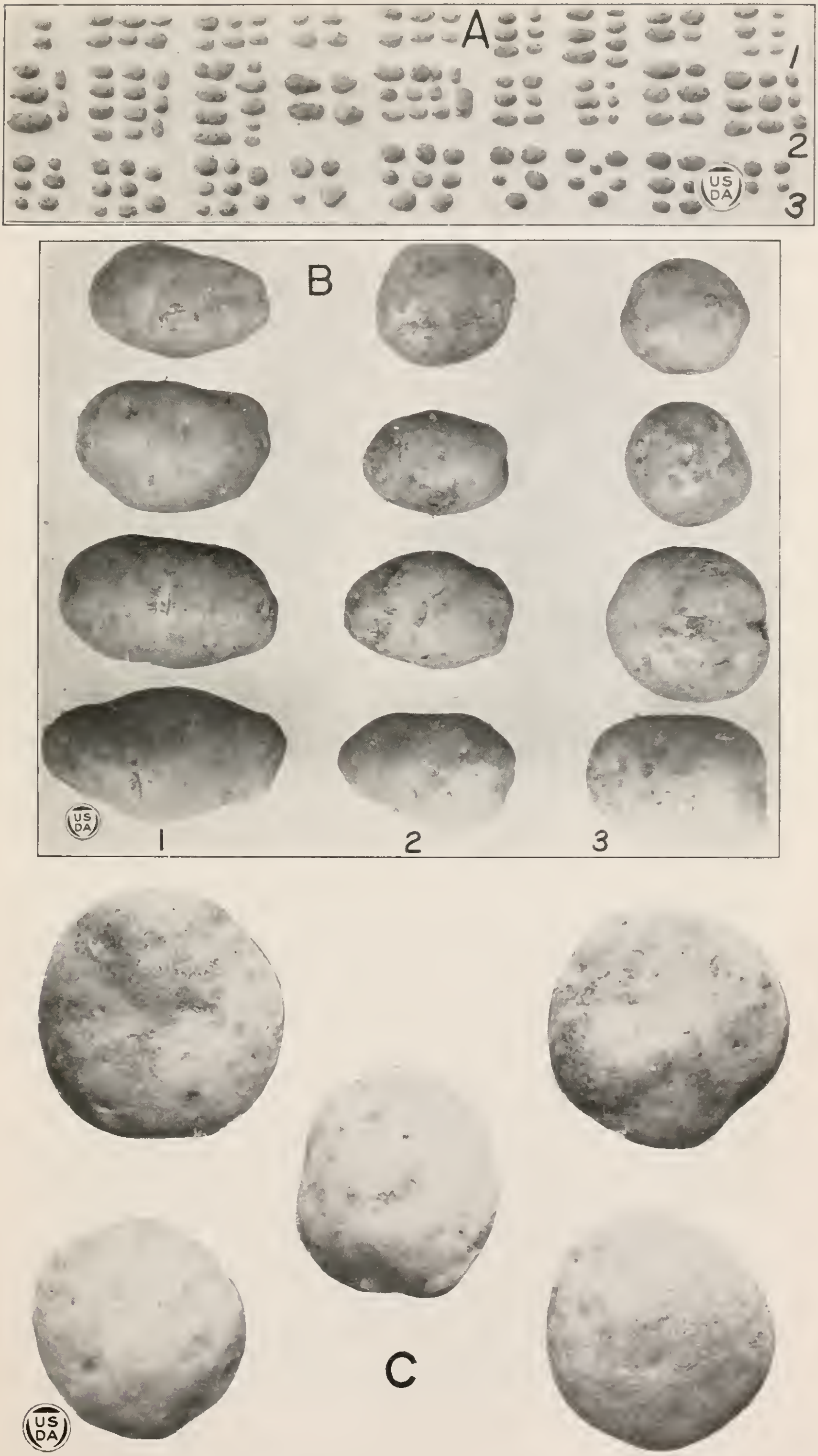
C.—(1) Six spindle-shaped tubers, progeny from the spindling tuber half grafted on the normal half tuber which produced the tubers shown in C, 2. (2) One hill spindling-tuber progeny from a normal half-tuber seed piece grafted on spindling tuber half-tuber seed piece. (3) Normal progeny from a quarter-tuber seed piece from the same tuber as the progeny in the middle row. Green Mountain variety.

PLATE 9

A.—Results of 10 of the spindling-tuber grafts. (1) Upper row, groups of tubers dug from 10 diseased hills grown from grafted half tubers originally diseased. (2) Middle row, groups of tubers dug from corresponding hills infected by grafting, grown from grafted half tubers originally healthy. (3) Lower row, groups of tubers from 9 hills grown from ungrafted quartered parts of the same tubers producing the middle row; shape healthy.

B.—Results of leaf-mutilation inoculation in insect cage, 1922. (1) Tubers from spindling-tuber hill serving as source of inoculum; (2) tubers from inoculated hill, with three tubers showing the disease; (3) tubers from uninoculated hill, control in same tuber unit as center hill but in different insect cage. Green Mountain variety. From stocks caged preceding year.

C.—Short tuber from spindling-tuber hill, with characteristic eyes and skin of the disease (center) and four tubers from healthy hills. Green Mountain variety.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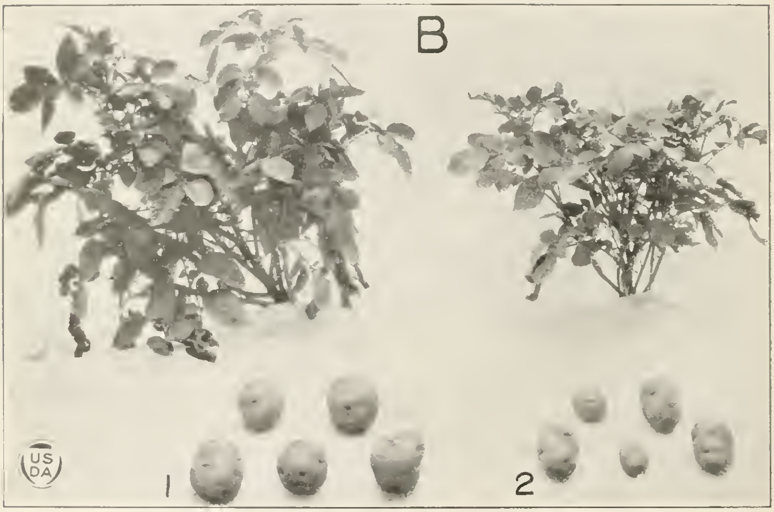
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C

118[10]

PLATE 10

A.—(1) Dwarfed Irish Cobbler plant. (2) Unmottled curly-dwarf Green Mountain hill diseased as result of aphid inoculation in preceding season from parent of plant (A, 1).

B.—(1) Healthy plant and corresponding tuber progeny. (2) Plant and corresponding tuber progeny infected with spindling tuber. Healthy and diseased plants from Rose 4 variety.

C.—Parts of the same Green Mountain strain. In left center, spindling-tuber seed potatoes planted. On right, all-mosaic seed potatoes planted. August 20, 1918.

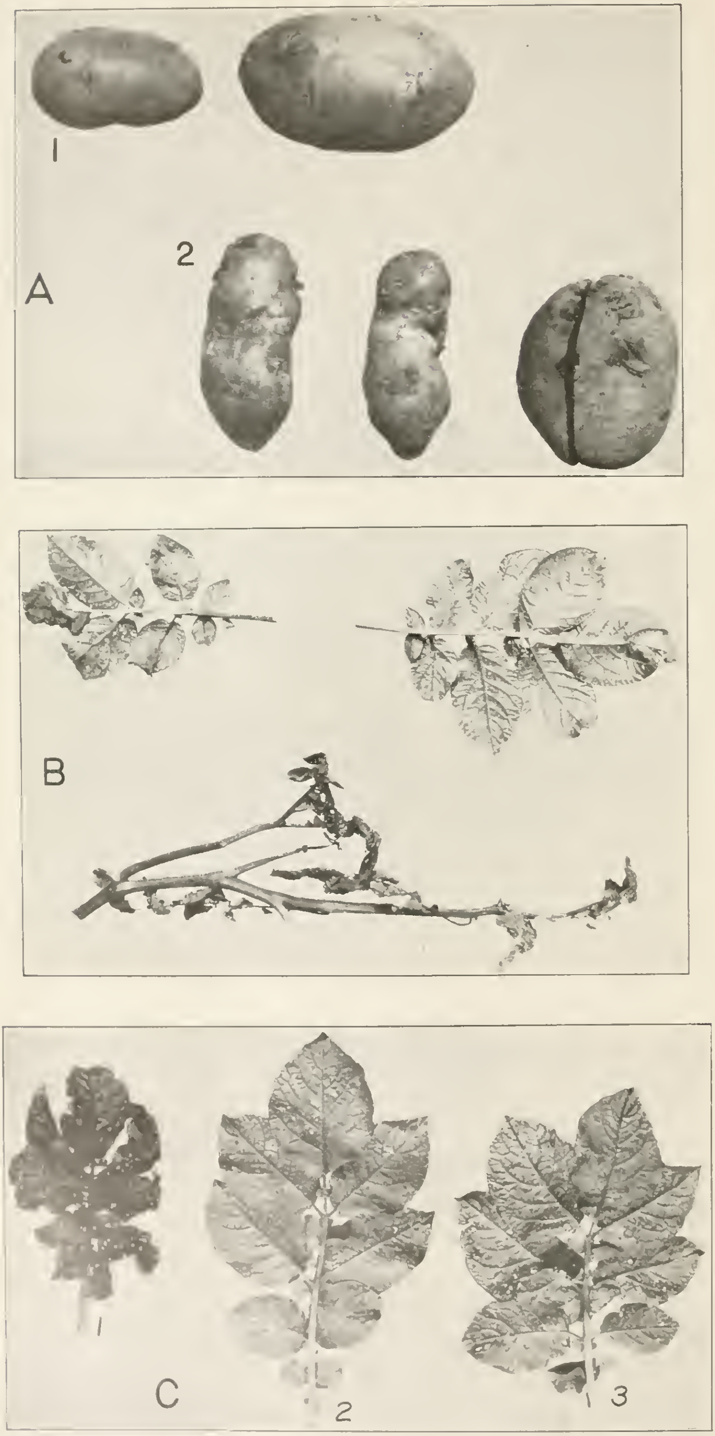
PLATE 11

A.—Healthy branch on an unmottled curly-dwarf plant. Variety Green Mountain.

B.—(1, 4) Healthy, and (2, 3, and 5) unmottled curly-dwarf plants, variety Green Mountain. Infection produced in first generation by leaf mutilation with juice from unmottled curly-dwarf Irish Cobbler.

C.—Foliage from Green Mountain plant showing streak.

118"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118

PLATE 12

A.—(1) Second-generation progeny to Green Mountain plant treated as A, 2. (2) Split tuber from Green Mountain plant inoculated by leaf mutilation with juice from unmottled curly-dwarf Green Mountain. The two somewhat spindle-shaped tubers are progeny from the split tuber.

B.—Leaves from Green Mountain plant infected with streak; stem from unmottled curly-dwarf Green Mountain plant, August 19, 1921.

C.—(1) Leaf from plant inoculated with juice from curly-dwarf Rural New Yorker. (2) Healthy leaf. (3) Leaf showing streaking. All leaves from Rural New Yorker variety.

PLATE 13

A.—Effects of recent leaf-mutilation inoculation, before appearance of disease symptoms.

B.—Capillary-tube inoculation with mosaic.


A
US DA


B
US DA


A


B


C

118[14]

PLATE 14

A.—Stem-in-vial inoculation with mosaic.

B.—Four Irish Cobbler hills, July 6, 1922, each with spindling tuber. The two on the left have had it at least a year longer than the two on the right, as the former were planted with seed tubers showing the disease while the latter were planted with seed tubers appearing healthy. The two outside plants have leaf roll in addition.

C.—Volunteer potato plants in buckwheat. July 3, 1921, in northeastern Maine.

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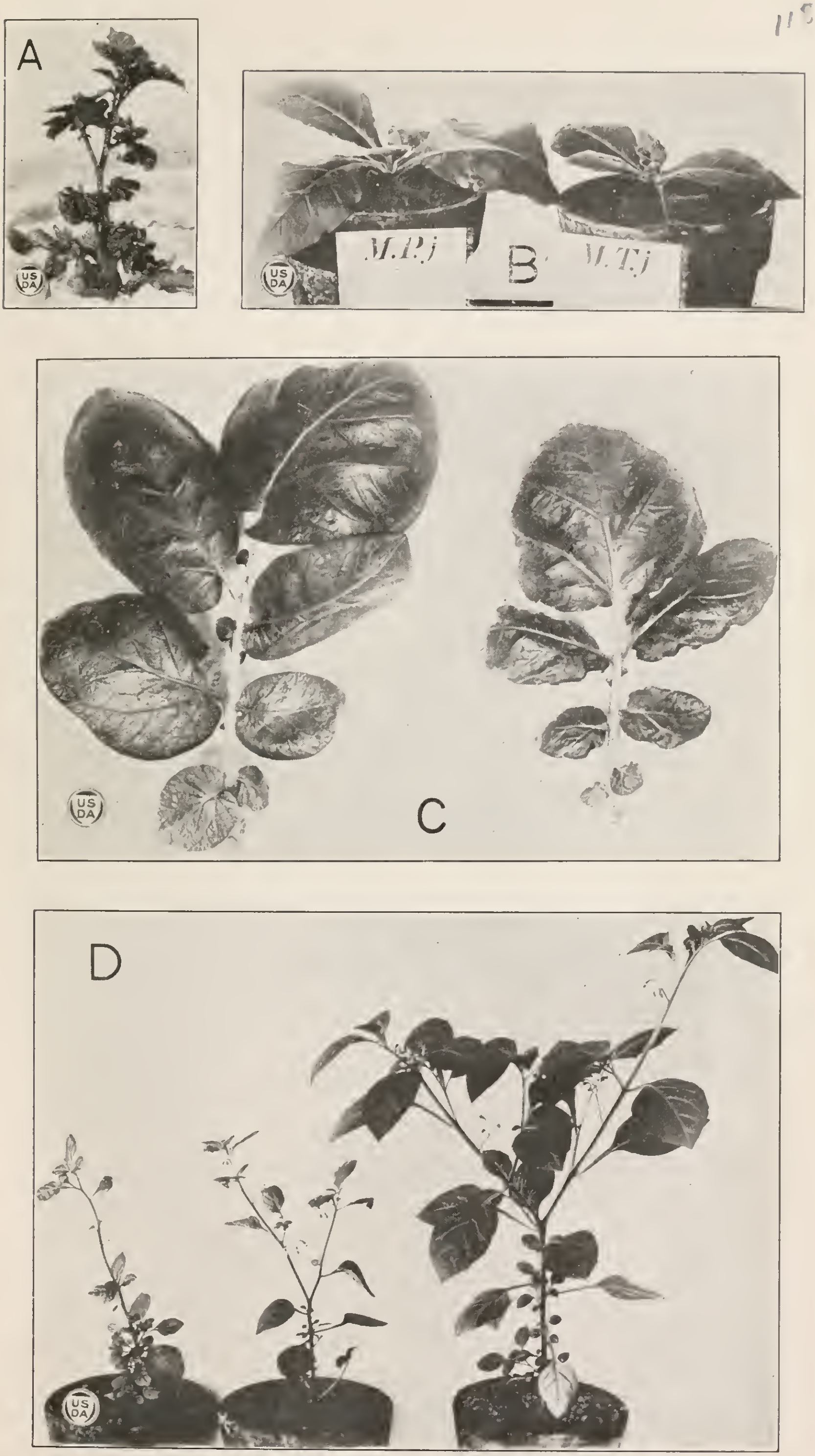
PLATE 15

A.—Green Mountain diseased plant representative of many that showed greater dwarfing and curling of the middle leaves than of the lowest or upper leaves, apparently as a result of certain weather conditions (July 2, 1921).

B.—(MTj) Tobacco plant inoculated 15 days previously and infected with juice from mosaic tobacco plant. (MPj) Tobacco plant of same seedling lot inoculated in same way with juice from mosaic potato plant 26 days previously, but not becoming infected. Orono greenhouse, March 2, 1920. From series 6 and 12, respectively, of Table XXI.

C.—Lower leaves of Bliss Triumph plants in same mosaic tuber unit. Larger leaf grown in warm room, smaller one in cool place. Wrinkling and mottling were more pronounced in cool place.

D.—Nightshade, *Solanum nigrum* L. On left, inoculated and infected with juice from a mosaic potato plant. In center, inoculated and infected with aphids from a mosaic potato plant. On right, uninoculated healthy control from same lot. Note that the lower leaves from the main stem are similar for the three plants, but that infection dwarfed the upper parts. Orono greenhouse, April 20, 1920.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D

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SOME RELATIONS OF THE CROWNGALL ORGANISM TO ITS HOST TISSUE[1]

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INTRODUCTION

For a number of years crowngall has been reported to be a limiting factor in Wisconsin in the cultivation of certain cane fruits. A recent survey by Jones (*2*)[3] revealed its presence in practically all the commercial cane fruit plantings in the State, and confirmed the earlier judgments as to its economic importance. Consequently, the writer undertook an investigation of this disease in the summer of 1920, with the aim of directing his efforts primarily along lines which promised to lay the foundation for more effective control measures.

In the early stages of this work it was decided to conduct preliminary studies upon the tomato, since it can be grown easily in the greenhouse in winter and is very susceptible to crowngall. While tomato stems were being inoculated by punctures, it was noticed that a water-soaked area promptly appeared about the point of entry of the needle.

It seemed evident that this darkened area, which tended to be parallel to the long axis of the plant, was caused by the occupancy of the intercellular spaces by liquid (Pl. 1, F, G). Sometimes, as seen from the surface, this water-soaked area attained a length of more than a centimeter, being especially conspicuous if the plant was quite turgid. No particular significance was attached to this observation until it was noted that the subsequently developed tumor appeared to conform closely in outline with this water-soaked tissue. Inoculations were then made in which the outlines of the water-soaked regions were marked with India ink. When the galls developed, they were found to coincide almost exactly in outline with the marked areas (Pl. 1, G, H). These results did not seem to accord with the generally accepted idea that the gall developed as a result of the stimulus from organisms which had gained entrance to the interior of the cells (*p. 5, p. 2; 9, p. 287; 10, p. 419*). The observed facts seemed rather to indicate the possibility that the organism began its activities in the liquid which occupied the intercellular spaces around the puncture.

[1] Accepted for publication May 2, 1923.

[2] The writer takes pleasure in expressing his indebtedness to Dr. L. R. Jones and Dr. G. W. Keitt, of the University of Wisconsin, and also to other members of the faculty, especially Dr. S. Eckerson and Dr. E. J. Kraus for many valuable suggestions in the prosecution of these studies.

[3] Reference is made by number (italic) to "Literature cited," p. 132.

Since further preliminary experiments confirmed these earlier observations, it was decided to extend the scope of the investigation to include a study of some of the relations of *Bacterium tumefaciens* Smith and Town., to its host tissues before proceeding with applied aspects of the problem. A report on this work follows.

The crowngall organism for these studies was secured at Madison, Wis., in 1920, from a gall found on a Kansas raspberry. Isolations were made by the poured plate method from portions of the gall which had been sterilized on the surface with mercuric chlorid. After the parasite was isolated, its pathogenicity was proved on both tomato and raspberry. Three successive platings and reisolations from the original culture of the organism made the author reasonably confident that he was working with a single strain. Repeated inoculation experiments have shown it to be actively pathogenic as evidenced by the production of characteristic galls. Cultural studies (in which the author is indebted to Dr. L. K. Jones for cooperation) have shown that it corresponds with Smith's description of the crowngall organism (*12, p. 105–127*), as isolated from the daisy, in all but a few respects.

The size limits of the raspberry organism on agar cultures were found to be 1 to 1.6 $\times$ 0.4 to 0.7μ as compared with 1 to 3 $\times$ 0.4 to 1.8μ for the daisy organism (*12, p. 106*). The size of the majority was found to be 1.1 $\times$ 0.5μ compared with 1.2 to 2.5 $\times$ 0.5 to 0.8μ for the daisy organism. The raspberry organism turned litmus milk pink slowly, and remained viable on culture media as long as six months, while the daisy organism never turned the litmus milk pink and lived only four or five weeks on culture media. These differences are not considered to be of practical importance. Smith (*12, p. 127–132*) has noted variations as great as these between different strains of crowngall bacteria. The writer, therefore, considers that he is dealing with the crowngall organism, *Bacterium tumefaciens*.

MODE OF ENTRY OF THE ORGANISM INTO THE HOST TISSUES

Many writers on crowngall have stated that wounds are necessary for the initiation of infection. In order to determine whether or not the organism could enter without the intervention of wounds, the following experiment was performed.

On the stem of each of 2 dozen tomato plants 10 droplike masses from a pure dextrose agar culture of the raspberry strain were applied with a camel's-hair brush. Six more plants were treated in a similar manner, but in addition a puncture was made into the stem through the center of each mass. After four weeks not a single gall had developed from the 240 masses where there were no wounds, whereas 100 per cent infection had appeared in the case of the parallel inoculations accompanied by punctures.

This experiment was supplemented by one in which tomato seeds which had been sterilized on the surface with 1 to 1,000 mercuric chlorid solution were planted in three 2 by 10 inch glass tubes partly filled with sterilized soil and in two containing synthetic agar. When the plants had grown sufficiently, the crowngall organism was introduced to both the agar and soil in large quantities in a way that precluded the possibility of wounding the experimental plants in the process. At the same time, two of the plants were inoculated near the apex of the stem to test their susceptibility. The inoculations were made on

December 15, 1920. On February 21, 1921, those that had received the inoculation at the top showed well-developed tumors. No other trace of gall could be found on any of the plants. Pure cultures of the organism were reisolated from both the soil and the agar. These readily infected other tomato plants when introduced into them. It appears, therefore, that infection did not occur in the tomatoes from organisms in the soil or agar in the absence of wounds.

Ten inoculations were made with a hypodermic syringe into the hollow pith cavities of castor bean petioles and stems. Varying amounts of a suspension of the organism ranging from 0.2 cc. to 1 cc. were injected. On October 4, 1922, three months later, these inoculations were examined. Well-developed galls were found at the punctures, but nowhere else, even though in some cases the path of the inoculum as it flowed down the pith cavity was traceable by a more or less dark stain. Plates were poured from this discolored region. The organism appeared in abundance and its identity was subsequently established by infection of tomatoes. Repetition of these experiments gave similar results. This work simply confirms the conclusions of earlier writers, namely, that under ordinary circumstances wounds are necessary for crown-gall infection.

Since wounds appear to be necessary for infection, it seemed desirable to inquire into the relation of size or character of the wound to gall development. This seemed especially pertinent in its possible relation to the associated water-soaking of the tissues. Glass and quartz rods were drawn out to make needles of different sizes ranging from 30μ to 385μ in diameter. A series of punctures were made with these needles through masses of the gall organism into tomato stems. After 25 days galls had developed, the diameters of which bore a more or less direct relationship to the diameters of the punctures (Pl. 1, A to E). Since the larger punctures released more liquid into the intercellular spaces, it appears that the size of the gall may be roughly proportional to the volume of tissue in which the intercellular spaces are flooded with liquid. A repetition of this experiment gave similar results.

The experiments outlined above and others of like import raised the question whether the inoculum must be inserted into the injured tissue or if the organisms might enter from the surface through the wounds. Consequently, 50 inoculations were made by puncturing through masses of bacteria which had been applied to tomato stems with a camel's-hair brush. In a parallel series, 50 punctures were first made with a sterile needle, and the organism was applied with a brush a few minutes later. Another series in which 50 sterile punctures were made in a similar manner, but without the application of any bacteria, served as controls. In this experiment not more than 10 punctures were made on a single plant. After five weeks galls had developed at every puncture where the organisms were applied while none had appeared on the controls. The developing galls showed no difference in relation to the two methods of inoculation.

The forces which govern this entry of the organism into the tissue are not definitely understood. The bacteria might conceivably be influenced by any or all of such factors as the collapse of drying tissue, negative pressure, sap rise, and motility with or without a chemotactic stimulus.

To test the possible relation of chemotaxis, very small capillary glass tubes were filled with expressed tomato stem sap. These were placed

in a hanging-drop preparation of a suspension of actively motile crowngall organisms in sterile distilled water in a manner similar to that described by Pfeffer (*4*). In a few minutes the bacteria were observed collecting in the tube. At the end of a half hour the motile organisms were thickly concentrated in the tube, while no active ones were observed outside. This experiment was repeated eight times with tubes of different diameters. In addition, it was tried with the positions of the bacterial suspension and the tomato sap reversed. In every case the motile bacteria collected in the liquid expressed from the tomato stem. It appears, therefore, that this tomato sap exerts a positively chemotactic stimulus upon the crowngall organism, which alone may be sufficient to account for the entrance of the bacteria into a puncture.

How long one of these individual punctures might serve as an infection court seemed an important point. One hundred and fifty punctures were made into tomato stems, and at intervals of time bacteria were applied to the punctures with a camel's-hair brush. The plants remained in an open greenhouse throughout the experiment. The results, recorded at the end of three weeks, appear in Table I.

TABLE I.—*A summary of results of experiments on the length of time that a puncture may serve as an infection court for B. tumefaciens*

Number of punctures.	Time between puncture and application of organism.	Infection after 3 weeks' incubation.	
		Number.	*Per cent.*
10	5 minutes	10	100
10	2 hours	10	100
10	4 hours	10	100
20	1 day	19	05
20	2 days	12	60
10	2½ days	9	90
10	3 days	2	20
10	4 days	2	20
10	5 days	0	0

Under more favorable conditions, when the plants were placed in a moist chamber for 24 hours following the application of the bacteria, 5 infections were secured from 10 punctures that were six days old and 2 infections from 10 punctures that were seven days old. Eight and nine-day-old punctures produced no galls. A repetition of this experiment gave similar results.

Although it is evident that conditions are important in determining the length of time a puncture may serve as an infection court, these experiments show that infection is very readily accomplished within the first day, and that it may under favoring conditions take place when the application of the organisms is delayed until as long as seven days after wounding the tissue. These results appear to be incompatible with the conception (*6, p. 170*) that the bacteria first establish themselves in wounded cells. It seems unlikely that in the case of such delayed infection any cell walls which might have been punctured at the time of the original wound would still offer open channels for the penetration of the bacteria into the living cells. Before seven days had elapsed the injured cells probably would either have died or healed the ruptures, provided they were capable of the latter.

For the purpose of determining whether punctured cells were necessary for the development of a tumor, the following experiment was devised. Thirty inoculations were made into tomato stems in the usual manner. After 24 hours a red hot needle was passed through the stem in as nearly as possible the position occupied by the inoculating needle. This treatment killed not only the cells that had previously been injured but also all those around the puncture for approximately 1 mm. in every direction. Sixty more inoculations were treated in the same manner, except that the burns were made at the end of an hour. In each case 30 controls were burned. After two weeks 100 per cent infection was found developing above and below the burned inoculations, while the controls showed no signs of proliferation (Pl. 1, I to K).

This experiment was repeated on 40 more punctures, with the variation that the bacteria were applied with a camel's-hair brush to the surface of the burn. Here also 100 per cent infection was secured above and below the burn. It seems unlikely that any cells which had been able to survive the heat could have had injuries in the walls through which the bacteria might have entered. These experiments indicate, therefore, that the bacteria exert their influence from some position outside the cells. This position, to begin with, at least, is probably in the liquid which penetrated the intercellular spaces following the puncture.

MIGRATION OF THE ORGANISM

In order to understand more clearly the relations of the organism to the liquid in the intercellular spaces, it seemed advisable to make observations on its motility both in water and in expressed plant juice.

For studies in water, bacteria from a two-day agar culture were mounted in sterile distilled water in a hanging-drop preparation. Observations were made on the length of time it took motile bacteria to move across the field of the microscope. No correction was made for the deviation of the bacteria from a straight line. The average of a dozen measurements showed them to move at approximately the rate of 1 mm. in one minute. Repetition of these measurements gave confirmatory results.

Similar tests were made in which expressed tomato sap was substituted for sterile distilled water. The motility of the organism did not appear to be materially changed so long as the preparations were fresh. However, the organisms in the tomato sap retained their motility longer than those in the sterile distilled water.

This gives an easy explanation of how the organisms might reach the limits of the region flooded with the liquid which was released by the puncture. Further studies of the range of migration were made in relation to extensive wounds caused both by mechanical crushing and by freezing.

Large wounded areas were produced on the stems and petioles of seven tomato plants by pressure from a glass rod. These bruised areas were made on one side of the stems and extended for longitudinal distances varying from 4½ to 10 cm. Crowngall bacteria were then introduced by a needle puncture into the lower portion of the injured region of five of the plants, while two were retained as controls. After four weeks, continuous well developed galls had extended for several centimeters above the puncture that had received the bacteria (Pl. 2, A, B). In no

case did the galls extend materially into the unbruised tissue. No proliferations were noted in the controls. In one case, a more or less continuous gall developed over the whole length of the crushed tissue. In the other four, the tumors extended over only about two-thirds or three-fourths of the length of the wounded area. In these cases it seemed possible that the bacteria failed to reach the limit of the crushed region because their path was obstructed by some break in the continuity of the liquid. A repetition of this experiment gave similar results.

Confirmatory evidence was obtained when the wound was produced by freezing. For this purpose, a carbon dioxid tank was provided with a single jet, from which the gas was projected against the tissue. By this means each of six plants was treated so that one side of the stem was frozen for a longitudinal distance of from 4 to 6 cm., with the result that a water-soaked area extended over this distance. Bacteria were then applied through a puncture at the lower end of the water-soaked area. After 18 days galls had developed at intervals all along the frozen areas (Pl. 2, C), but the more prominent proliferations were produced at the points of puncture. Controls showed no such proliferation. No evidence of any tumor strands could be found between the galls at the places of puncture and the other tumors by a study of free-hand sections.

These experiments indicated that the bacteria could travel several centimeters at least if they were given a continuous channel of fluid through which they might pass. This raised the question of whether or not they might pass through the tracheae.

Consequently, a tomato stem was heavily inoculated in one place in such a manner that the punctures passed through in several directions so that the penetration of some of the tracheae appeared certain. Then with a sterile needle, punctures were made through the stem at intervals from the apex down to the point of inoculation with the expectation of penetrating some of the same vessels that were injured at the point of inoculation. The plant wilted as the result of such treatment, but recovered after a day. In three weeks well-developed galls were found at the point of inoculation, and smaller ones were observed at irregular intervals in two-fifths of the sterile-needle punctures (Pl. 2, H, I). The farthest one was at a distance of 7 cm. from the point of inoculation. These results are interpreted to indicate that those punctures about which no galls were produced had not penetrated vessels which contained bacteria. The same method was tried with six other plants. Two of these gave results similar to those just described, but the other four developed proliferations only at the points of inoculation. Control plants which were punctured but not inoculated produced no gall formation.

A further study of the passage of the organism through stem tissues was begun in the laboratory by placing a tomato stem which had been cut under water in a suspension of a pure culture of the bacteria (Pl. 2, D). After an hour and a quarter the stem was cut at intervals of 5, 3, 2, and 1 cm. above the suspension. Sap was pressed out from the cut base of each section that was removed and transferred to agar, where the gall organism developed in abundance. Smears of the sap from 5 cm. above the suspension showed large numbers of the organism. This experiment was repeated five times on tomato and once on raspberry with time intervals varying from 30 minutes to 3 hours and with variations up to 9 cm. in the distance above the point of entry of the organisms. In every case the organism was easily recovered.

In order to determine more clearly whether the bacteria had passed up through the tracheae, a tomato stem was tied to a supporting rod and then a section several centimeters long was frozen by means of carbon dioxid. On the next day the soft tissue had collapsed and dried, leaving apparently only strands of dead conductive vessels with the firm covering of shrunken tissue about them. This treatment did not cause severe wilting of the upper part of the plant, especially when part of the top was pruned off. The tissue which had been frozen was allowed to dry an additional day to further guard against the occurrence of a moist passage for the bacteria outside of the vascular tissue. Then the stem was cut off and placed in a suspension of the bacteria (Pl. 2, E), as previously described. Two hours later the bacteria were recovered in large numbers from the interior of the stem above the collapsed area, but not in such great abundance as in the earlier experiments. This may have been due to a reduction in the flow of sap through the bundles.

These experiments were modified so that the stem could be cut part way through under a suspension of the gall organism. For this purpose a cylinder of cork was divided in two and fastened around the stem with pins. Upon this as a base was placed a short piece of large rubber tubing which had been cut down the side for convenience in locating it around the stem. The whole was sealed with vaseline so that it formed a cup about the stem (Pl. 2, F). This was filled with a suspension of the organism and a transverse incision was made in the stem below the surface of the fluid. The suspension and cup were removed at the end of 5 hours. After 48 hours punctures were made in the stem with the tip of a sterile knife at intervals from the top down to the cut. Eight weeks later four galls had developed out of eight punctures. One of these was 8 cm. above the inoculation cut. Four repetitions of this experiment gave like results. They produced, in order, three galls from 6 punctures, eight from 14, six from 11, and seven from 9.

While one may not be fully confident of the exact course followed by the bacteria, these experiments suggest that, under favorable conditions, the crowngall bacteria can pass through the tracheae and induce galls at some distance from the point of entry, if they are provided with an avenue of escape from the vessels and suitable circumstances for development. However, there is no evidence to show that they can produce galls if they remain inside these vascular elements.

To determine whether or not the organism occurs commonly in some of the vascular tissue, 60 sterile punctures were made in the stems of tomatoes above three-weeks-old galls. Four weeks later not a single one showed any evidence of tumor formation. So it may be assumed that this parasite may travel in some parts of the vascular bundles if conditions are favorable, but that probably it does not ordinarily induce infection from this position.

EARLIER ATTEMPTS TO LOCATE THE ORGANISM

The experiments so far described have indicated that the crowngall organism begins its relations with its host tissues in the liquid occupying the intercellular spaces about the wounds. The first striking evidence in support of this conception came to the writer's attention in an experiment described on an earlier page. There it was shown that the galls developed rather uniformly throughout the water-soaked area produced by the puncture and closely coincided with it in outline. So from the

beginning it appeared to be the simple and natural suggestion from this evidence that the bacterial invasion not only started in the intercellular spaces but possibly also continued therein in a manner comparable to that of various other bacterial pathogens.

With this question in mind a search was instituted with the hope of detecting the bacteria in situ. In the beginning it was realized that the organism might be located only in certain portions of the gall and might also be more apparent at one time in the stages of development than at another. Consequently, in this search the efforts were not confined to any portion of the stem taken at one time, but were directed to whole sections of stems with galls in different stages of development.

Material was killed in such fixatives as chrom-acetic, Flemming's, Carnoy's, picro-formal, formal-alcohol, and Benda's. Paraffin sections were then cut and stained with carbol-fuchsin, methylene blue, gentian-violet, Haidenhain's iron-alum-haematoxylin, safranin, rose Bengal, Giemsa, neutral red, orange G., and light green, either alone or in combination. No satisfactory demonstration of the organism in the tumor tissue was secured. The organisms could not be distinguished, except as Smith states (*11, p. 17*), relatively near the entrance of the needle. However, it was noted that the bacteria could be traced from the puncture out through the intercellular spaces for a short distance (Pl. 3, C), and could also be located in many of the intercellular spaces above or below the puncture. This was quite easy in the cortex and pith where the intercellular spaces were large. Search was of course persistently made for evidence of the occurrence of bacteria within the cells, but they were detected only in those cells which were close enough to the puncture to be subject to injury. In such cases the organisms were present in sufficient quantities to occupy a major portion of the cells (Pl. 3, D). It appears, therefore, that if entrance into the cells is provided for the bacteria, they collect there in considerable numbers. It seems quite improbable, however, that cells showing such abundant invasion should survive.

Efforts were made to locate the bacteria with gold chlorid (*7, p. 127*) and with Gram's stain, employing amyl alcohol as used by Smith (*11, p. 18–19*), but these also gave unsatisfactory results. Such combinations as ruthenium red and methylene blue, used so successfully by Jones (*3, p. 332*) were equally ineffective.

While studying paraffin sections the writer noticed that certain of the walls bounding intercellular spaces took the stain much more deeply than others. This was found to be true of the spaces that had been filled with liquid after the puncture and to which the bacteria had gained access (Pl. 3, A). The bacteria seemed to have exerted an action on the walls which made them stain more deeply. This influence was noticed to extend a short distance into the middle lamella (Pl. 3, B), but its exact range has not been determined. This seemed to be in accordance with the hypothesis that the bacteria were located in the intercellular spaces and possibly to some extent in the middle lamellae. If this were true, a satisfactory explanation would be given alike for the different types of staining shown by different cell walls, and for the development of galls from the region water-soaked by the puncture.

So the problem became one of trying to differentiate between the cell wall, which had been made more sensitive to stain by the action of the bacteria and the organisms themselves. For this purpose sections were

used which showed the bacteria in mass about the puncture. In this case the differentiation was not so difficult because the bacteria were together in large numbers and the walls in these early stages had not undergone any change due to the bacterial action. But this gave no idea of the relation of small numbers of bacteria to the walls that took the stain more deeply.

Since the usual paraffin method had yielded inconclusive results for the writer, as it had earlier for Smith (*8, p. 253*), it seemed evident that some variation in method must be employed if the organism were to be demonstrated in the tissue. At the suggestion of Doctor Eckerson a variety of microchemical tests were performed on free-hand sections of gall tissue in the hope that either the walls or protoplasm of the bacteria could be found to give a characteristic reaction. For the wall the chitosan reaction was tried, but it gave only unsatisfactory results. For protein, the biuret, Berlin blue, and xanthoprotein reactions and Millon's reagent gave no better success, so far as demonstrating the bacteria was concerned.

While these reactions were being tried, observations were made on free-hand sections of tomato galls that varied from one day to three weeks in age. These sections were mounted in water in the usual manner and examined under an oil immersion lens. In the middle lamella, especially of the collenchymal cells, small bacteroid bodies were observed (Pl. 4, A), which, however, varied considerably in size. At the same time, in certain of the intercellular spaces, bacterium-like bodies were observed which were of rather uniform dimensions. These were associated with a yellowing of the adjacent cell walls. They were visible, just as bacteria are in hanging-drop preparation, because of the differences in light refraction. Occasionally, also, as Smith observed (*11, p. 17*), bacteria could be seen in cut cells. However, the fact that they appeared inside the open cells was no proof that they were present in this position before the section was made. If they were in the intercellular spaces, one would expect some to escape as soon as the wall was cut, and then they might appear anywhere on the surface of the section.

This possibility that the bacteria might wash out of the middle lamellae or intercellular spaces into the water during the sectioning made it seem advisable to cut the material dry. The actual practice, with a good sharp razor, proved to be much easier than anticipated. The sections were mounted quickly in a small drop of water, in glycerin, or in lacto-phenol (equal parts of lactic acid, phenol, glycerin, and water). This last medium was the most convenient because it did not dry out like the water, nor did it contain so many annoying air bubbles as the glycerin. At the same time it proved to be an excellent preservative.

The large bacteroid granules observed in the middle lamellae especially between the collenchymal cells were found in observations made in February, 1922, to be associated with the rapidly growing gall tissue but not with the more slowly growing uninoculated tissue (Pl. 4, A, B). However, when an examination was made in June, 1922, while the tomato stems were growing rapidly, similar granules were found in the normal as well as the gall tissue. Their presence seems to be associated with the rate of growth. Polarized light showed them not to be doubly refractive. They lost their identity quickly in 3 per cent hydrochloric acid upon the application of heat, and slowly in the lacto-phenol mounting fluid. They stained both with methylene blue and ruthenium red.

Together with the middle lamellae they were dissolved after treatment with dilute acids followed by dilute alkalies. It appears that they are composed of some pectic substance, possibly calcium pectate.

LOCATION OF THE BACTERIA IN THE INTERCELLULAR SPACES

Evidence has been accumulated which shows that the bacterial granules which were observed in the intercellular spaces bordered by yellowed walls are the crowngall bacteria in situ. These bacterial bodies have been observed to be constantly associated with crowngall tissue in tomato stems through four series of inoculations in which the galls were examined at two-day intervals from the day of inoculation until the galls were 22 days old. At the latter age they showed the characters of maturity—that is, they had well-developed hypertrophic and hyperplastic areas which were abundantly supplied with new vascular elements. The bacterial bodies were found very easily in the early stages and without much difficulty in the later ones because of the yellowing of adjacent walls which accompanies their presence. This yellowing is similar to that which may be observed in connection with various types of injured tissue. Consequently, although crowngall bacteria in the intercellular spaces appear to be accompanied by a yellowing of the surrounding walls, this color does not always indicate the presence of bacteria.

With the aid of polarized light it was observed that the yellowed walls had lost their property of double refraction. It appeared that the bacteria had produced some change in the cellulose of adjacent walls. However, a further consideration of the nature of this action is beyond the scope of this paper.

The phenomena just described were not observed in either sound tissue or tissue that had been punctured but not inoculated. The most likely sources of confusion were found to be the granules of pectic substance already described and deposits of very tiny crystals of calcium oxalate. These latter were observed quite commonly in the gall tissue. Their identity as crystals was very easily established by the use of polarized light.

After the location of the bacteria was ascertained the method of demonstrating them in the paraffin sections became simplified. It appeared that the customary methods had failed because the bacteria produced an effect on the cell wall that made it take up the stain more heavily than did the normal walls. This resulted in a masking of the bacteria when they were not present in very large numbers. It was found also that the intercellular spaces occupied by the bacteria gave a positive protein reaction to Millon's reagent, while the bacteria themselves did not. Whether this substance is produced by the bacteria or by the neighboring cells is not understood, but its presence certainly renders more difficult the demonstration of the bacteria.

It remained to discover a combination of stains that would color the bacteria and still not hide them by staining too deeply the walls and protein substance surrounding them. This was found when dilute carbol-fuchsin was used in combination with light green. Tissue which had been killed in chrom-acetic or formal-alcohol fixatives was dehydrated and embedded in the usual manner. Sections were cut between 6 and 12μ in thickness. The sections were stained for one minute in carbol-fuchsin which had been very greatly diluted (1 part by volume of carbol-fuchsin to 100 parts of water). Then after very rapid treatment with

absolute alcohol the sections were cleaned and stained by a saturated solution of light green in clove oil, rinsed in xylol, and mounted in balsam.

Slides made in this manner showed the walls acted on by the bacteria and the xylem walls to be stained red, while the rest of the tissue appeared green. The bacteria for the most part appeared red along with the bordering tissue (Pl. 3, C, D; 4, C, D; and 5, A–D). The crystals of calcium oxalate remained uncolored. The previously mentioned granules of pectic substance were never observed in stained preparations.

The bacteria found in these sections are present in larger numbers than was previously supposed. Repeated statements have appeared in the literature that the organisms were scarce (*12, p. 193; 11, p. 18*). These appeared to be confirmed by the writer's earlier isolations. In view of the comparisons of microscopic and plate counts made from soil by Conn (*1, p. 10*), it seemed logical to expect a poured plate to show the presence of no more than one-tenth of the number of bacteria that might be distinguished under the microscope. But even with this discount the results from earlier platings showed a comparatively small number of colonies.

The chance examination of a 10-day-old isolation plate revealed an interesting phenomenon. This plate had been poured from gall tissue which had been treated with mercuric chlorid and washed and crushed before mixing with the agar in a manner similar to that described by Smith (*12, p. 22*). A bacterium-free zone surrounded the portions of tissue for a radius of more than a centimeter, while outside of this region a very large number of colonies had appeared. These were proved by successful inoculation into tomato to be the gall organisms. It seemed quite clear that the treated tissue had an inhibitory influence on the bacteria, due either to the diffusion of the disinfectant used or to some detrimental product of its own.

To determine whether or not a short treatment of the tissue with the mercuric chlorid was inhibiting to the growth of the bacteria, three plates were poured from a suspension of a pure culture of the crowngall organism at a dilution which would produce about 1,000 colonies in each plate. Small pieces of normal tomato tissue which had been dissected out under aseptic conditions and treated with mercuric chlorid were placed in the first and second plates. In the first the tissue was placed 45 seconds in mercuric chlorid 1 to 1,000, as Smith (*12, p. 24*) describes and washed a minute and a half in sterile water, while in the second the treatment with the disinfectant lasted 3 seconds and the washing 10 seconds, as recommended later by Smith (*10, p. 434*). The tissue in the third plate was not treated with the disinfectant, but was placed in sterile distilled water for 30 seconds. After five days the portions of tissue which had been treated with mercuric chlorid for either period had bacterium-free zones around them for a radius of about 1½ cm. Outside of these the bacteria appeared in great abundance. The other fragment, which had been treated only with water, appeared to have exerted no inhibitory effect on the organisms. This experiment was repeated twice with the variation that gall tissue was used as well as normal tissue. The results were confirmatory in every respect.

A few contaminating organisms were secured in the plates with the gall tissue. Some of these were able to tolerate the inhibiting influence.

They apparently diminished the toxic action of the mercuric chlorid sufficiently to permit the crowngall colonies to grow around them. So, occasionally, in the clear zones surrounding the treated gall tissue, a contaminator appeared, surrounded by a small zone of colonies of the crowngall organism.

The presence of a large number of causal organisms in crowngall tissue was demonstrated by dissecting out and grinding in white sand and water, under aseptic conditions, a 2 mm. cube of young gall tissue which was produced by inoculation. Dilution plates were poured of the suspension secured. Counts and computations indicated that this block of gall tissue contained approximately 50,000 bacteria capable of producing colonies. Another similar cube was found to contain about 30,000. Under different conditions a piece of gall tissue ¼ c. mm. in volume ($1 \times 2 \times 0.125$ mm.) was dissected out under sterile conditions and crushed in a Petri dish. Over 1,100 colonies of the gall organism developed, with practically no contamination. Doubtless many more would have appeared if the plate had not been so crowded. Other similar pieces have produced colonies ranging in number from 18 to 11,600. When these figures are multiplied by 10, one secures numbers consistent with those indicated by the microscopic examinations.

Although the crowngall bacteria have been found in larger numbers than has commonly been supposed, a very wide range of variation has been observed. The number of bacteria secured in culture seems to depend on the number of intercellular pockets of bacteria that are broken open so as to release the organisms. Since the number of these pockets varies with the age of the gall, the portion of the gall selected for isolation, the conditions of growth, etc., considerable differences are to be expected in the numbers of bacteria that appear in plates or sections.

Since it was discovered that mercuric chlorid exerted this inhibiting influence, its use has been abandoned in routine isolations for the crowngall organism. A procedure like the following has been found successful. A 2 to 3 mm. cube of young gall tissue is dissected out under aseptic conditions. This is dropped into 10 cc. of sterile distilled water and crushed. Then dilution plates are poured from the suspension.

A further check on the accuracy of the interpretation of the previously described intercellular bodies as the crowngall bacteria was made by observing their multiplication from free hand sections of gall tissue, subsequently by isolating them from such preparations, and by establishing their identity with the usual cultural and inoculation methods. This was accomplished in the following manner.

Free-hand sections of crowngall tissue were cut under aseptic conditions and mounted in drops of melted nutrient dextrose agar on thin cover slips. After the agar had solidified, each cover slip was fixed with vaseline on a van Tieghem cell as in the preparation of a hanging-drop. When the bacteria developed, observations made at about eight-hour intervals showed that the colonies were unusually definitely localized on account of the solid medium. No difficulty was experienced in examining the preparation even with an oil-immersion lens. Colonies were observed to grow consistently from the previously described intercellular bacterial pockets. When the examination was completed, usually when the preparations were two or three days old, the sections in agar were crushed in separate tubes of sterile distilled water, from which dilution plates were poured. These plates have commonly yielded prac-

tically pure cultures of the crowngall bacteria, which were identified by successful inoculations.

From the foregoing observations and experiments we believe it may safely be concluded that the crowngall bacteria are located in certain intercellular spaces of the host tissue, and that they are present in larger numbers than has been supposed.

No attempt has been made in this paper to describe the responses of the host to the bacteria in the intercellular position. These activities, with special emphasis on the formation of the "tumor strands," will be treated at a later time.

SUMMARY

(1) Crowngall infection in tomato stems was found to take place only through wounds.

(2) It was not found to be necessary for infection that the organisms should be carried into the tissue at the time of puncture. Successful results followed their application to the surface of wounded tissues. They were observed to have a positively chemotactic response to expressed tomato sap, which could account for their entrance following surface application. Under favorable conditions infection was induced by organisms applied to the surface as long as seven days after the puncture.

(3) When needle punctures were made into turgid tomato and tobacco stems, the intercellular spaces above and below for several millimeters became occupied by liquid and appeared water-soaked. When puncture inoculations were made, the galls which developed were found to coincide closely in outline with these water-soaked regions.

(4) Galls of different sizes were induced experimentally by varying the sizes of the inoculating needles and consequently the extent of the water-soaked areas.

(5) A half hour after inoculations were made in the usual manner by punctures, a red-hot needle was inserted in the path of the inoculating needle. It is believed that this destroyed all the cells that had previously been ruptured. The subsequent development of galls from the outer margin of the water-soaked region is interpreted as indicating that the bacteria are not dependent upon gaining access to ruptured cells, and is, on the other hand, consistent with the idea that they are distributed in the liquid in the intercellular spaces.

(6) When a continuous channel of liquid was provided in the tomato stem either by mechanical pressure or by freezing, the bacteria migrated and produced galls several centimeters from the point of inoculation. The subsequent gall development occurred over the area approximately corresponding to this original water-soaking of the tissue.

(7) When the organisms were introduced into a wound which cut some of the vascular bundles, they appeared to travel in some part of the conductive tissue, probably the tracheae, and when a wound enabled them to escape, induced proliferation. This type of infection is not considered a common occurrence in nature.

(8) The usual methods of staining failed to demonstrate the bacteria in situ. This appeared to be due to the staining of the adjacent cell walls and of the substance which occurred in the intercellular spaces which was of similar intensity to that of the bacteria, thus masking them.

(9) The bacteria were observed in unstained free-hand sections and also in paraffin sections stained with very dilute carbol-fuchsin and light green. Apart from the region immediately around the wound, they were found only in an intercellular position. They were seen in gall tissue of all ages from the day of inoculation until the gall showed the characters of maturity about 24 days after inoculation.

(10) Isolation and microscopic observations indicated that the bacteria are present in larger numbers than was previously supposed. The treatment of the tissues with mercuric chlorid before plating appears to lower the count so as to give a false impression of the number of bacteria present.

(11) A further control on the location of the crowngall bacteria was made by observing their multiplication in free-hand sections cut under aseptic conditions and embedded in agar, by subsequently isolating them from such preparations, and by establishing their identity with the usual cultural and inoculation methods. Dilution plates poured from such sections have repeatedly yielded thousands of colonies of the crown-gall organism with less than one per cent contaminations.

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PLATE 1

Stems of tomato and tobacco from inoculation experiments, showing the relation o crowngall development to the size of punctures and to the extent of the water-soaked areas surrounding the wounds [1]

A, B, C, D, E.—The relation between the diameter of the inoculating needle and the size of the gall. Photographed 25 days after inoculation. The diameters of the inoculating needles used, expressed in microns, were as follows: On A, 30; B, 56; C, 147; D, 248; and E, 385. Note that the size of the resulting galls increases quite consistently with the size of the needle and the extent of the water-soaked area. ×11/20.

F.—Longitudinal section of a tobacco stem showing the flooding of the intercellular spaces with liquid after a puncture. The water-soaked area (b) appears as a dark region about the puncture (a). ×4/5.

G.—Tomato stem showing water-soaked areas resulting from punctures with inoculating needle (outlined with India ink). Photographed promptly after puncturing and making. ×9/10. (The stems G to K, inclusive, show the relation of crowngall development to the water-soaked areas surrounding the wounds.)

H.—A stem treated as G, photographed three weeks after inoculation. The ink lines (retraced in order to appear distinct) show that the margins of the resulting galls coincide approximately with the limits of the original water-soaked area. ×9/10.

I.—A stem that was punctured and, after a half hour, burned in the same place with a red-hot needle. No bacteria were applied. It served, therefore, as a control on J and K. ×9/10. (It appears in I, J, K that galls may develop from water-soaked areas in which the ruptured cells have been destroyed by heat.)

J.—As I, except that the crown-gall bacteria were applied immediately after the punctures were made, that is, a half hour before the burning. Galls appeared in the water-soaked regions that were unaffected by the burning. I and J were photographed two weeks after the beginning of the experiment. ×9/10.

K.—As J, but photographed three weeks after inoculation. ×9/10.

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[1] These results are interpreted to indicate that the crowngall bacteria are located in the liquid which is released by the wound and which occupies the intercellular spaces about the punctures.

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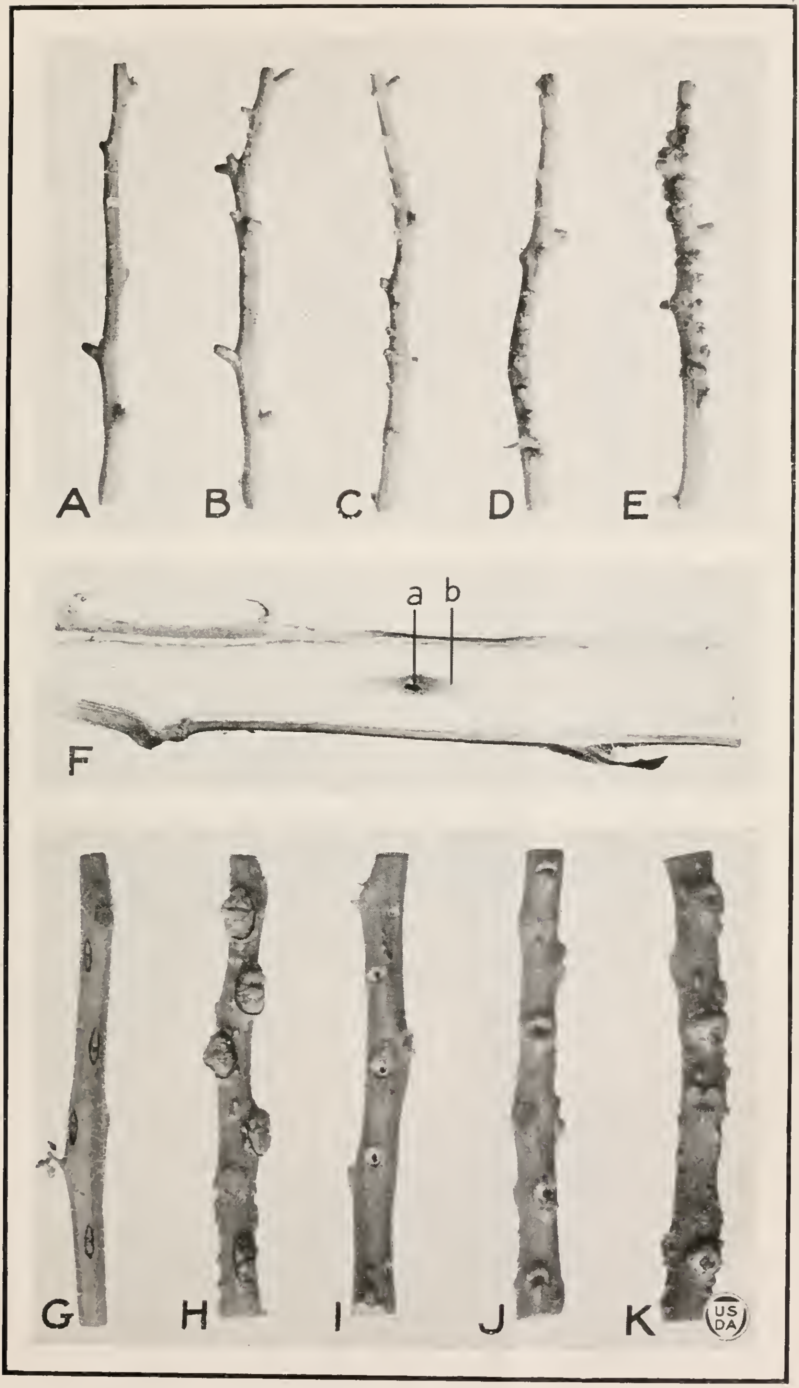


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PLATE 2

Tomato stems from inoculation experiments with reference to the migration of the crowngall organism in the tissues

The stem A to C, inclusive, shows that the bacteria traveled through regions water-soaked by injury and produced galls some distance from their point of entry. In all cases the stems were inoculated only at a.

A, B.—The stems were bruised from b to c and inoculated at a. Proliferations developed over the entire length of the injured area. ×2/3 (approximately).

C.—The stem was frozen from b to c with carbon dioxid and inoculated at a. Galls appeared at intervals. ×2/3 (approximately.)

The figures D to G, inclusive, show that the bacteria may travel through some portion of the vascular system.

D.—A section was cut out of the stem and the base of the upper part was submerged in a suspension of the crowngall bacteria. The organisms were recovered by cultural methods at intervals up the stem, which suggests that they passed up through the tracheae. ×1/5 (approximately).

E.—As D except that the stem was frozen and dried with the aim of preventing the passage of the bacteria up the stem except through the dead vessels. The bacteria were recovered in culture from the stem above the frozen region, indicating that they had passed through some part of the vascular tissue. ×1/5 (approximately).

F.—A cup, in which a suspension of bacteria was placed, was made from cork, rubber tubing, and vaseline. An incision was made in the stem under the surface of the suspension and sterile cuts made above and below. ×1/5 (approximately).

G.—A stem inoculated as in F. The cup surrounded the stem at d. The sterile cuts which produced galls appear at b. It seems likely that the bacteria which produced the galls at b passed through some part of the vascular tissue. ×2/5 (approximately).

H, I.—Stems severely wounded and inoculated by punctures which ruptured some of the vascular bundles at d and a. Sterile punctures were made at intervals above. Galls developed at b which were produced by bacteria which probably passed through some of the vascular elements, and were permitted to escape by the punctures. H, ×2/5 (approximately); I, ×1/2.

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PLATE 3

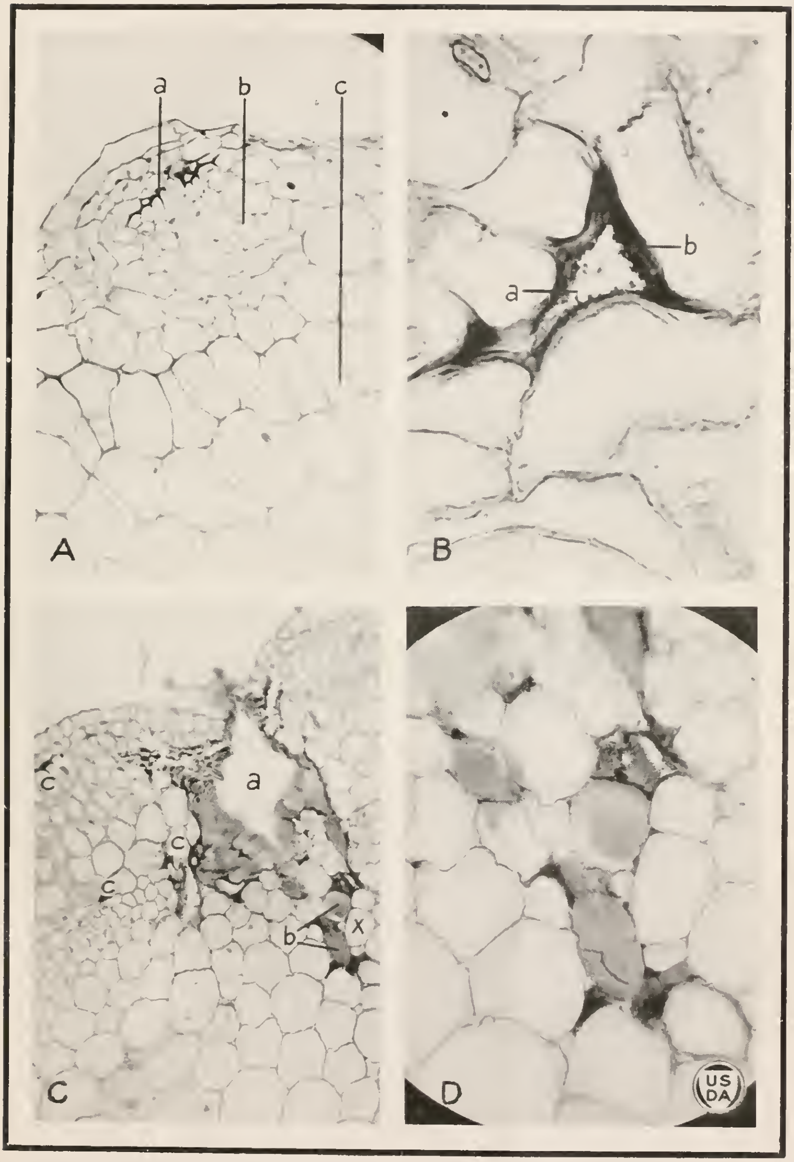
Photomicrographs of crowngall tissue from tomato stem

A.—Six days after inoculation. The darkly stained region (a) which contains the bacteria is surrounded by a more or less circular hyperplastic area (b), and this is bordered by a region of hypertrophy (c). Paraffin section, stained with Flemming's triple stain. ×167.

B.—A darkly stained region on the same slide as section A, but a few sections removed, is shown at a higher magnification. In this preparation the bacteria (a) are masked by the increased intensity of staining in the wall (b) and the substance in the intercellular space. ×600.

C.—Forty-eight hours after inoculation. The large clear space (a) shows where the needle passed. This is surrounded by masses of bacteria which appear also in the injured cells (b) and intercellular spaces (c). The region at x is enlarged in figure D. Paraffin section stained with dilute carbol-fuchsin and light green. ×134.

D.—An enlargement of the region marked x in figure C. Ruptured cells appear so crowded with bacteria that their continued activity seems unlikely. ×400.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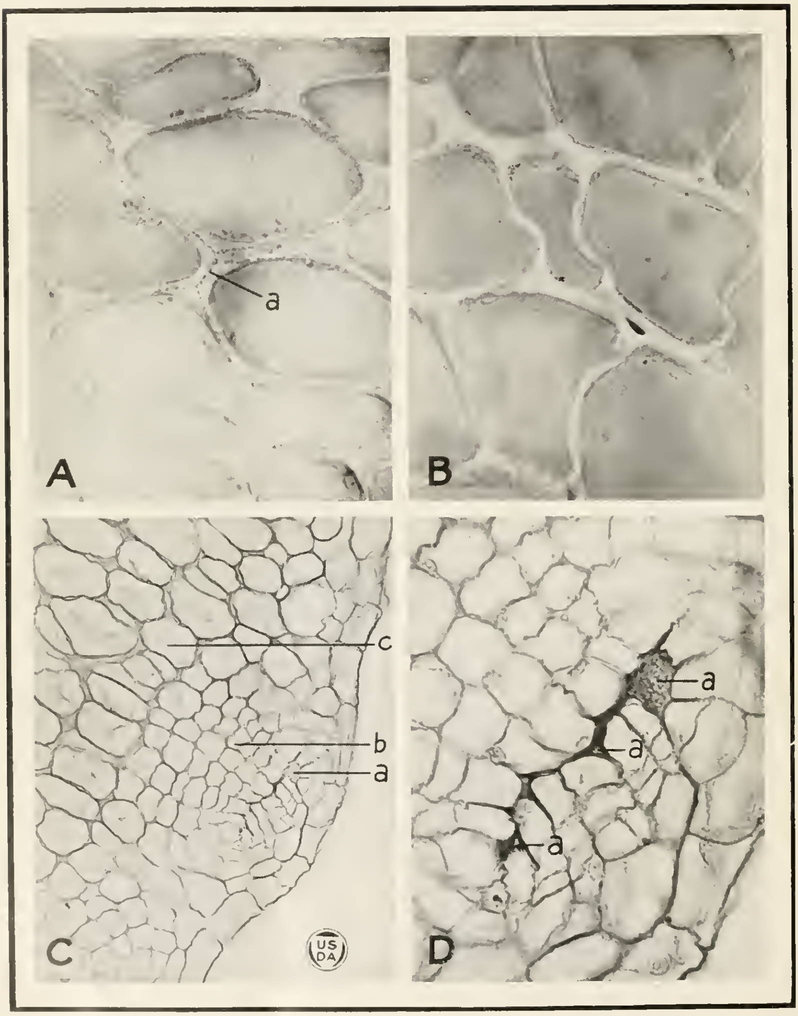
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PLATE 4

Photomicrographs of pectic granules in the middle lamellae and of crowngall bacteria in the intercellular spaces of gall tissue

A.[1]—Four-day-old gall. Pectic granules (a) are visible in the middle lamellae between large cortical cells. They have been observed in rapidly growing uninoculated tissue in July. Free-hand section, unstained. ×600.

B.—As A, except taken from an uninoculated plant grown in the greenhouse. ×634.

C.—Eight-day-old gall. The intercellular spaces (a) are filled with bacteria and surrounded by newly formed regions of hyperplasia (b) and hypertrophy (c). The region containing bacteria is enlarged in figure D. Paraffin section stained with dilute carbol-fuchsin and light green. ×200.

D.—An enlargement of the region containing bacteria in figure C. The intercellular spaces which contain the bacteria are maked a. In the slide these walls and the bacteria are stained red while the rest of the tissue shown appears green. ×634.

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[1] A and B were photographed in March, 1922.

PLATE 5

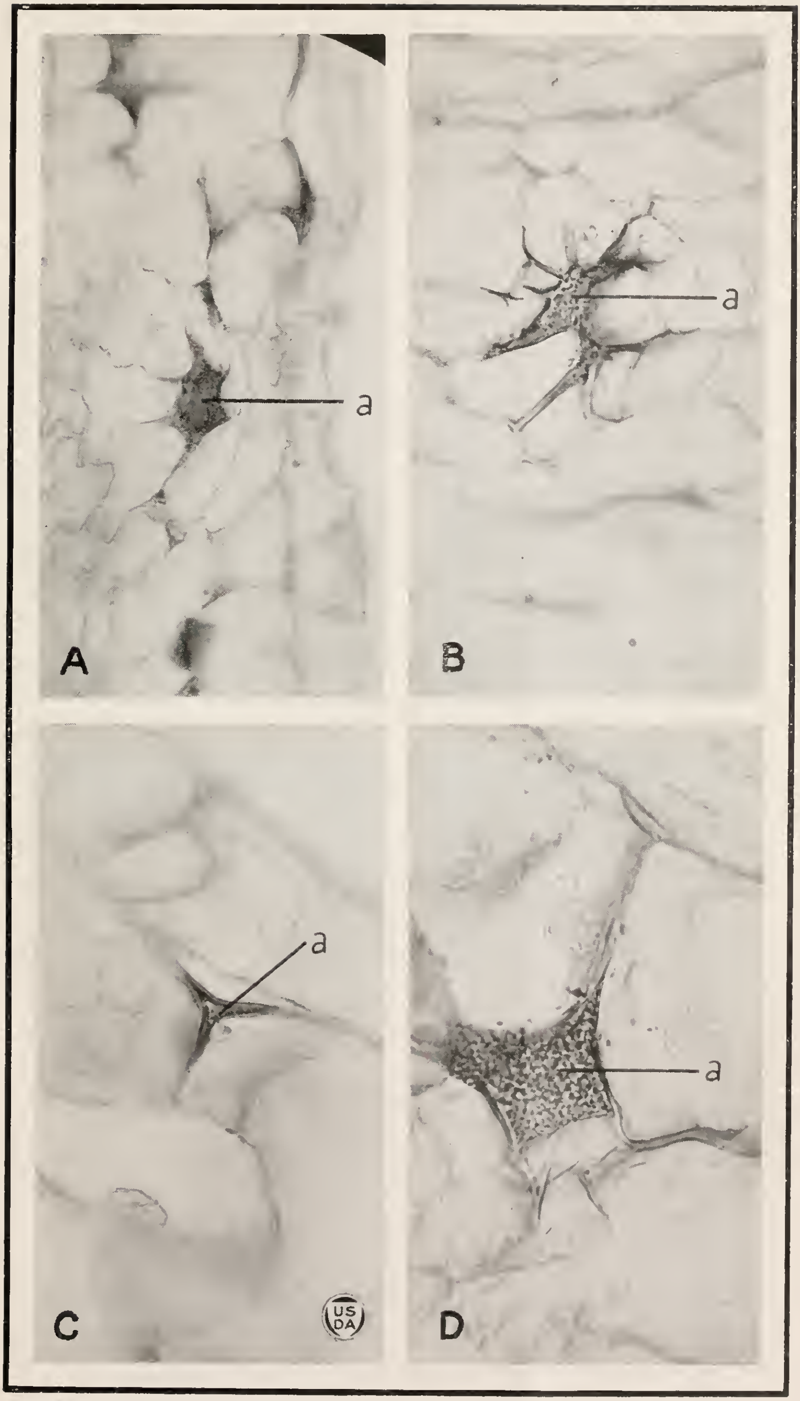
Photomicrographs of the crowngall bacteria in the tissue

A.—Six-day-old gall. The bacteria are seen in the intercellular space marked a, from which they extend a short distance into the middle lamellae. Paraffin section stained with dilute carbol-fuchsin and light green. ×555.

B.—As A, except from a 10-day-old gall. ×917.

C.—As A, except from a 14-day-old gall. The bacteria have acted on the wall so as to produce a thickening and a change in standing reaction. ×835.

D.—As A, except from a 14-day-old gall. ×917.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133

OXYGEN-SUPPLYING POWER OF THE SOIL AS INDICATED BY COLOR CHANGES IN ALKALINE PYROGALLOL SOLUTION[1]

By LEE M. HUTCHINS, *Pathologist, Fruit Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture,* and BURTON E. LIVINGSTON, *Professor of Plant Physiology, Johns Hopkins University*

INTRODUCTION

It is generally agreed among students of plant physiology and agriculture that the roots of many plant forms require free oxygen, obtained from the surrounding soil, for their healthy activity. The careful experimental work of Free, Cannon, and others, as well as the general experience of agriculturists and horticulturists, indicates that many kinds of plants suffer markedly and even die when access of free oxygen to their roots is cut off. One of the reasons given for cultivating the soil in agricultural work is that tillage tends to facilitate the entrance of atmospheric oxygen into the soil in which the roots are found. It is often observed that low areas of a field of grain, for example, collect water, which stands on the surface of the ground for some time after a rain, and the poor plant growth frequently noticed in such areas—even after the surface water has disappeared—is often explained by supposing that the flooding of the soil hindered or prevented the downward movement of oxygen from the air.

From considerations of this kind it is at once suggested that, if health is to be maintained, the roots of any individual plant must receive elementary oxygen from the surrounding soil at a rate sufficiently rapid to keep their physiological processes adequately supplied. As is indicated by the experiments of Livingston and Free[2] it is to be expected that this necessary rate of oxygen supply will be found to be different for different kinds of plants and probably for different plants of the same kind grown under sufficiently different conditions. It would then follow that a plant in an otherwise suitable environment would remain healthy only so long as the conditions in the soil about its roots were such as to allow the necessary rate of oxygen absorption through the root surfaces. The health of the plant would be impaired if the ability of the surrounding soil to supply oxygen to the roots were sufficiently diminished so that the rate of arrival of oxygen at the root periphery fell below the rate of absorption necessary for healthy activity. It seems clear that a given root surface can not absorb oxygen any more rapidly than this substance comes to it from the surroundings.

The ability of soil to supply oxygen to an oxygen-absorbing surface, such as that of a root, might be expected to be greater with relatively dry and loose soils, and especially near the soil surface, while it would be less for the more compact or wetter soils and for greater depths. This oxygen-supplying power of the soil may sometimes be related, in a general way, to the oxygen content of the soil in the vicinity of the absorbing

[1] Accepted for publication May 2, 1923.

[2] LIVINGSTON, B. E., and FREE, E. E. THE EFFECT OF DEFICIENT SOIL OXYGEN ON THE ROOTS OF HIGHER PLANTS. *In* Johns Hopkins Univ. Circ. 293, p. 182–185. 1917.

surface, but it is surely not generally proportional to the oxygen content of the soil in general at the given depth; a soil containing very little oxygen might still deliver oxygen to the absorbing surface at a considerable maintained rate, while a soil containing much oxygen might soon become depleted near the absorbing surface so that its maintained rate of delivery would be low. Assuming that roots require an oxygen supply from the soil, the oxygen condition that determines whether they shall be healthy or unhealthy is therefore not to be defined in terms of the oxygen content of the soil at the various depths where the roots occur. The soil feature that determines the health of the roots, as far as oxygen is concerned, must be the oxygen-supplying power. It should be noted that, like other environmental conditions, this dynamic feature of the plant's subterranean environment ought to be a limiting condition only when its value is lower than the necessary rate at which oxygen absorption must proceed if the plant is to remain healthy. As long as the soil is able to supply oxygen more rapidly than it is required by the roots, it should make no difference—other conditions being adequate for health—how great the oxygen-supplying power may be.

It seems that ecology and agricultural science would be advanced if we might be able to study and compare the oxygen-supplying powers of field soils at various depths, down to the lower limit of penetration by the roots of plants growing in them. The dynamic soil feature here emphasized has apparently not yet attracted the attention of ecologists, foresters, and students of crop plants; indeed, it seems only to have been barely mentioned in the literature thus far. It was somewhat surprising to find no mention of this dynamic consideration in such a thorough review of the literature of soil aeration as that recently made available by Clements's [3] excellent monograph on this subject. In the Year Book of the Carnegie Institution of Washington for 1921 there is a report by W. A. Cannon,[4] in which he says:

> It is the rate of supply and not the partial pressure of the gas (in the soil air) that is important.

Such studies and comparisons as those just suggested can not, of course, be begun until some suitable method has been devised for measuring the oxygen-supplying power of the soil, and the work here reported was undertaken for the purpose of testing certain suggested methods that seemed to have some promise in this direction. The results herein reported on the oxygen-supplying power of the soil were obtained during January and February, 1922.[5]

METHOD.

Any method for measuring the power of the soil to supply oxygen to an absorbing surface must fulfill two conditions: (1) The absorbing apparatus must allow oxygen from the surroundings to enter by diffusion and not by mass streaming (since there is no mass streaming of oxygen into plant roots), and the absorbing surface must not alter significantly, as to its ability to absorb oxygen, when the surrounding

[3] CLEMENTS, Frederic E. AERATION AND AIR-CONTENT, THE RÔLE OF OXYGEN IN ROOT ACTIVITY. 183 p. Washington, D. C. 1921. Bibliography, p. 163-183. (Carnegie Inst. Wash. Pub. 315.)

[4] CANNON, W. A. ROOT-GROWTH IN RELATION TO A DEFICIENCY OF OXYGEN OR AN EXCESS OF CARBON DIOXID IN THE SOIL. *In* Carnegie Inst. Wash. Yearbook 20 (1921), p. 48-51. 1922.

[5] These studies were carried out in the laboratory of plant physiology of Johns Hopkins University under the general direction of Dr. M. B. Waite, pathologist in charge, Office of Fruit-Disease Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

conditions change (otherwise the rate of absorption of oxygen could not be taken as a measure of the ability of the soil to supply this element); (2) the oxygen absorbed must be removed and collected in some way so that the amount taken in during a test period may be quantitatively determined.

After some preliminary experimentation a porous-porcelain cylinder closed at one end, such as is used in atmometry,[6] was adopted as a promising form of absorber. To prevent the streaming of the gas through the porous wall the pores were finally filled with paraffin oil (Nujol). As experience has shown, oil-impregnated porous porcelain seems to be adapted for the taking up of oxygen by diffusion. The cylinder was ground down until the lateral wall, excepting the enlarged rim, was from 1 to 2 mm. thick; the rim was then coated with sealing wax to prevent oxygen absorption in that region. The oil treatment was applied by filling the hot cylinder (about 100° C.) and allowing it to stand upright for 12 hours, after which the superfluous oil was removed.

The open end of the cylinder was closed by a rubber stopper holding two glass tubes, one of which extended through the cylinder nearly to the closed end, while the other terminated just inside the stopper. All joints were sealed with spar varnish, as was also done throughout the remainder of the apparatus which is described in the following paragraphs. Oxygen from the surroundings diffuses through the wall of the absorbing cylinder, tending to make the partial pressure of oxygen in the cylinder cavity equal to the external partial pressure in the immediate neighborhood. De-oxygenated gas (illuminating gas thoroughly scrubbed by alkaline pyrogallol solution, adopted merely because it was convenient and served well for these preliminary experiments, which did not deal with living plants) is passed slowly—60 cc. per hour—through the absorber, so that oxygen is removed from the cylinder as rapidly as it diffuses in from the outside. The internal partial pressure of oxygen is therefore always almost nil, while the instrument is in operation. The gas coming from the absorbing cylinder is conducted to a bottle containing the indicator solution, in which the oxygen is all absorbed and measured.

The indicator bottle is of the ordinary wide-mouth form, 4 cm. in diameter and 10 cm. high, closed by a sealed-in rubber stopper, through which pass the glass tubes for the entrance and exit of the gas. The entrance tube reaches to the bottom of the bottle, while the other extends only slightly beyond the stopper on the inside.

Ten cc. of the indicator solution is placed in the indicator bottle. For this solution, which collects the oxygen coming in the gas stream from the absorbing cylinder, and which gives indication of the amount of oxygen thus collected, an alkaline solution of pyrogallol is used. It is prepared as follows: Two stock solutions, one containing 50 gm. of pyrogallol dissolved in 100 cc. of distilled water, and the other having 450 gm. of potassium hydroxid in 500 cc. of distilled water, are mixed with oxygen-free water in the proportions of 1:2:20.

As is well known, an alkaline solution of pyrogallol absorbs elementary oxygen with great avidity, the absorption proceeding with marked rapidity and continuing when the gas is rapidly supplied until large amounts have been absorbed. If made under proper conditions, such a pyrogallol solution is practically colorless before any considerable amount

[6] LIVINGSTON, Burton Edward. THE RELATION OF DESERT PLANTS TO SOIL MOISTURE AND TO EVAPORATION. 78 p., 16 fig. Washington, D. C. 1906. Literature cited, p. 77–78. (Carnegie Inst. Wash. Pub. 50.)

of oxygen has been taken up, but with the beginning of oxygen absorption the solution begins to show color, and the color gradually darkens as the process is continued, passing through a series of tints and shades, from very pale brownish orange through more intense browns that become redder and finally somewhat purplish as the final color is approached. After much oxygen has been absorbed the solution is nearly opaque and almost black.

A rather prolonged study of the possibilities of using the rate of change in the color of the indicator solution just described, as an indication of oxygen absorption, led to the colorimetric method here used. An arrangement was finally devised by which the indicator solution may be examined and compared with standard color solution from time to time, as it gradually darkens during the period of the experiment. One or two standard color solutions, in bottles similar to the indicator bottle, are placed beside the latter for this comparison, and the examination is always made with transmitted light from a 60-watt Mazda incandescent electric lamp. The light reaches the bottles through a diffusing screen of white paper and the bottles and lamp are inclosed so that all light reaching the eye comes directly through the solutions to be compared. As the apparatus is usually operated, three bright windows are visible, each about 5 mm. high and 2 cm. wide, arranged in a horizontal row. The light of the middle window is from the indicator bottle, while that of either of the other two windows is from the corresponding color standard. By this arrangement it is not difficult to determine with great accuracy whether the indicator solution appears darker or lighter than any standard solution with which it is compared, and this determination can be made almost instantly at any time during an experiment.

After many unsuccessful attempts along other lines, Arny's plan [7] for preparing standard color solutions was tried and found to be adequate for the present purpose. Three solutions were prepared, a red one of cobalt chlorid, a yellow one of ferric chlorid, and a blue one of copper chlorid. By mixing these three solutions in proper proportions and with proper dilutions (as determined empirically) a number of permanently colored mixtures were secured, each one of which represents, with a high degree of accuracy, one of the colors traversed by the pyrogallol indicator solution during the early stages of its oxygen absorption. In the final experiments only two color standards were used. Standard A is very pale, and its color matches that of the indicator solution when a very little oxygen has been absorbed. Standard B is much darker, and was so chosen that its color matches that of the indicator solution at the end of an experiment period of convenient length. The compositions of the two standard solutions thus far used are shown in Table I. A 1 per cent solution of hydrochloric acid is used instead of water.

TABLE I.—*Composition of two standard color solutions*

Basic compound.	Pale standard solution (A), in 20.5 cc.	Dark standard solution (B), in 20 cc.
	Gm.	*Gm.*
Cobalt chlorid crystals	0.07	1.00
Ferric chlorid crystals	0.54	0.14
Cupric chlorid crystals	0.144	0.20

[7] ARNY, H. V., and RING, C. H. STANDARDIZED COLORED FLUIDS. *In* Jour. Franklin Inst., v. 180, p. 199-213. 1915.

In operation, the outlet tube from the absorbing cylinder is joined by lead tubing to the inlet tube of the indicator bottle, and the outlet of the latter is connected to the intake of an ordinary bottle aspirator. The outlet of the aspirator is joined to the inlet of a series of large scrubber bottles partly filled with alkaline pyrogallol, and the outlet of this series is connected by lead tubing to the inlet tube of the absorbing cylinder. The gas system is thus completely closed. All tubing excepting the connections to the absorbing cylinder is of glass, with rubber connections.

The aspirator consists of two 8-liter bottles, each closed by a sealed-in rubber stopper bearing two tubes, one of which reaches to the bottom of the bottle, while the other reaches only through the stopper. The two long tubes are joined together by a 2-meter length of rubber tubing. Each of the short aspirator tubes is connected by a Y and a 2-way cock to the closed circulatory system, so that either one of the short aspirator tubes may be operated as inlet to the aspirator while the other is operated as outlet. One bottle stands about 1.5 meter above the other, the upper one having been initially filled with water and the siphon started through the long tube. Water flows out of the upper bottle and enters at the bottom of the lower one. Gas is gradually removed from the lower and transferred through the circulatory system to the upper, the two-way cocks being properly set so that the gas circulates in the direction indicated above. When the upper one is nearly empty the positions of the two bottles are reversed and the two-way cocks are both reversed, so that circulation continues in the right direction. The rate of gas movement through the system is maintained practically uniform by adjusting the relative heights of the two aspirator bottles. A nearly closed cock was introduced into the circulatory system, adjusted to give the required rate of gas movement. The rate of movement was determined from time to time by counting the number of bubbles entering the indicator bottle per minute.

A safety bottle arranged like the indicator bottle was inserted in the system so that the circulating gas traversed it just before entering the absorbing cylinder, and another was inserted in the gas stream immediately beyond the indicator bottle. The alkaline pyrogallol solution in these safety bottles did not become colored, thus indicating that the gas entering the cylinder was without oxygen, and that no oxygen from the cylinder escaped being collected in the indicator bottle.

The apparatus was furnished with a somewhat complicated system of tubes, cocks, extra containers, etc., and with a Chapman filter pump to furnish suction when needed in this auxiliary system. By means of this system the two stock solutions—of aqueous pyrogallol and aqueous potassium hydroxid—were prepared and brought together with proper dilution, the indicator bottle and the safety bottles could at any time be emptied, rinsed and refilled with fresh indicator solution, and other necessary operations could be performed, all without the entrance of oxygen into the system at any place excepting via the absorbing cylinder.

The apparatus just described in its essentials was used as follows: The absorbing cylinder is placed in the exposure for which the oxygen-supplying power is to be determined, and the gas stream is started and allowed to continue for several hours, to establish dynamic equilibrium. A fresh charge of indicator solution is placed in the indicator bottle (the bottle is thoroughly rinsed several times with new indicator solution, after the old solution has been withdrawn, the old solution and the rinsing

portions being discharged through the waste conduit), and the color of this solution is compared with standard color solution A at frequent intervals until the two colors appear alike. When this occurs, the starting time of the experiment is recorded. The gas circulation continues at the established rate, and the indicator solution becomes gradually darker, until it matches standard color solution B. Then the time the experiment ends is recorded. The difference between these two time records gives the number of minutes required for the indicator solution to take up enough oxygen to alter its color from that of standard color A to that of B. Since all of this oxygen has come through the walls of the absorbing cylinder the time required for this color change may be taken as inversely proportional to the rate of entrance of oxygen into the cylinder. It is of course necessary to allow the gas circulation to go on for a considerable time—at least 3 to 5 hours—after every change in the exposure of the absorbing cylinder, in order to allow the cylinder and the gas spaces of the apparatus to come into dynamic equilibrium with the new surroundings. In practice, readings were generally taken before this equilibrium had become established, and the numerical results indicated the transition from the old to the new conditions of exposure. In order to avoid the accumulation of oxygen in the cylinder it has been found best to maintain the gas circulation through the system at all times, whether readings are being taken or not.

According to several approximate determinations the color change just mentioned required about 0.015 cc. of oxygen under the ordinary pressure and temperature conditions of the laboratory air. This constant of the apparatus has not yet been precisely determined, however, since its exact value does not enter into the problem dealt with, as will appear below. As far as tests have gone, with the various features of the apparatus as they were, it appears that small variations in the temperature of the laboratory were without influence upon the results.

EXPERIMENTATION WITH SOIL

In the following paragraphs will be described a series of experiments made with a box of garden soil in the laboratory. The results are to be regarded more as an illustration of the way in which the apparatus may be used than as quantitative data on the soil used. They do furnish interesting indications as to how the oxygen-supplying power of the soil may be expected to differ with different depths, states of packing, and moisture contents. The soil used was a loam, with considerable admixture of organic matter, and water enough to make it moist but not wet, such as is commonly used for potting greenhouse plants. The box used was of wood, 30 cm. wide, 40 cm. long, and 30 cm. deep, paraffined on the inside.

The absorbing cylinder with its two lead tubes (1.5 m. long) connecting it to the rest of the system, was first placed upright in a glass jar containing enough mercury to submerge the absorbing portion. The gas stream flowed for 48 hours without any alteration in the color of the indicator solution, showing that there were no appreciable leaks in the system. The mercury was then withdrawn from the jar, exposing the external surface of the absorbing cylinder to the air of the room. The rate of oxygen absorption through the walls of the cylinder under these conditions was rapid enough so that the color change from color standard A to color standard B required 40 minutes. From this it appears that

the cylinder had the power to absorb oxygen from the air at the rate of about 0.015 cc. in 40 minutes, or about 0.0225 cc. per hour.

The cylinder was next placed horizontally in the soil box on top of a layer of loosely sifted soil 8 cm. deep, after which more soil was loosely sifted into the box until the layer above the cylinder was 7.5 cm. deep. In placing the cylinder in the soil the lead tubes were bent so that they extended into the soil to the required depth and then, before reaching the absorbing cylinder, extended twice around it in the form of a horizontal ellipse lying about half way between the cylinder and the box wall. This precaution was taken so as to avoid possible direct movement of air from the atmosphere to the cylinder along the tube surfaces.

Within three hours of the time the cylinder was placed in the soil its rate of delivery of oxygen to the indicator solution showed a noticeable decrease, and during the next two days this decrease continued. At the end of that period the time required for the color change was 80 minutes; that is, the cylinder was absorbing at the rate of about 0.0112 cc. of oxygen per hour.

The cylinder was itself able to take up oxygen at the rate of about 0.02 cc. of oxygen per hour—as shown by the air test—and 8 cm. of very loosely packed moist soil could supply oxygen to the cylinder only about half as rapidly as the cylinder could have taken it up if the soil could have supplied it.

The cylinder was allowed to remain for 40 days in the soil, without disturbance, to allow some settling. The box was covered and no water was added during this period. Twenty-four determinations were then made during the next 71 hours, with color-change periods of from 68 to 83 minutes, the average being 75.8 minutes. This indicates an oxygen-supplying power of about 0.0119 cc. per hour. Eight cm. of loosely sifted soil was then added to the box, thus bringing the cylinder to a depth of about 16 cm. below the soil surface. A lengthening of the color-change period was evident after 3 hours, and the period lengthened gradually during the next 3 days, as shown by numerous readings taken at intervals. During the succeeding 2 days several series of readings were taken—16 in all—with color change periods of from 84 to 116 minutes, the average being 94 minutes. This indicates an oxygen-supplying power, at this depth, of about 0.0096 cc. per hour.

Water was next added to the soil, corresponding to 6 cm. of rainfall, which very nearly saturated the soil. This wetting produced shrinkage in the soil so that the soil surface came to be only 13.5 cm. above the cylinder. A marked increase in the length of the color-change period was shown after 8 hours. Five determinations during the next 18 hours gave periods of from 150 to 179 minutes with an average of 164 minutes, indicating an oxygen-supplying power of about 0.0055 cc. per hour.

The soil was next packed firmly, lowering the surface about 2.5 cm. but without disturbing the tubes or cylinder. A determination made directly after packing gave a color-change period of 240 minutes, and another determination immediately thereafter gave a period of 5,050 minutes. The last-named period indicates an oxygen-supplying power of only about 0.0002 cc. per hour.

For the particular cylinder used, it appears that, while the oxygen-supplying power at a depth of 8 cm. in loose moist soil was about 0.0119 cc. per hour, the corresponding power at a depth of 13.5 cm. of firmly packed, nearly saturated soil, was only about 1.5 per cent as great. Changing the exposure of the absorbing cylinder from a depth of 8 cm.

to a depth of 16 cm., without otherwise altering the soil, changed the oxygen supplying power from 0.0119 to 0.0096 cc. per hour for this cylinder. Wetting the soil diminished this value from 0.0096 to 0.0055 cc. per hour, and packing the wet soil diminished it from 0.0055 to 0.0002 cc. per hour.

The numerical results just given in terms of the lengths of the color-change periods and in terms of cubic centimeters per hour for the cylinder used, have been approximately calculated also to terms of cubic centimeters per hour per square meter, cubic millimeters per hour per square meter, and milligrams per hour per square meter. They are shown in all five different ways in Table II. Of course it is understood that these values are all very rough approximations, and they are rounded off to convenient decimals. The calculations have been made by assuming a temperature of 20° C. and a barometric pressure of 76 cm. of mercury. The different lines of the tabulation represent merely different ways of expressing the four different values dealt with.

TABLE II.—*Approximate results of oxygen tests on moist and wet soil*

	Moist, loose soil.		Wet soil.	Packed, wet soil
Depth (in soil) of absorbing cylinder (cm.)	8	16	13. 5	11
Color-change period in minutes	76. 0	94. 0	164. 0	5050. 0
Cc. per hour cylinder	0. 0119	0. 0096	0. 0055	0. 0002
Cc. per hour square meter	1. 3	1. 0	0. 59	0. 02
Cu. mm. per hour per square meter	1300. 0	1000. 0	590. 0	20. 0
Mgm. per hour per square meter	1. 73	1. 33	0. 79	0. 03

The results of this preliminary study indicate, as was to be expected, that the oxygen-supplying power of the soil for a plant root becomes less (1) as the root lies deeper in the soil, (2) as the moisture content of the soil above the root increases, and (3) as the soil above the root becomes more firmly packed. It may perhaps be estimated that the oxygen-supplying power of the soil about the roots of ordinary agricultural plants may be something like 1 per cent as great when the soil is packed and saturated by heavy rains as it is when the soil has recently been tilled and is not excessively moist.

CONCLUSION

As stated in the introduction, the aim of this preliminary study was to test certain suggested and seemingly promising methods of approach toward the measurement of the oxygen-supplying power of the soil at different depths and under different conditions of soil moisture and of packing. The writers believe that this aim has been attained and that the method described in the foregoing pages may be regarded as at least fairly promising for this kind of measurement. Doubtless many improvements will be made and other methods may be devised, based on the same or different principles and procedures. This paper is to be regarded merely as a report of progress—enough, however, to show very clearly that the dynamic soil feature here considered will not prove to be unusually difficult of measurement whenever ecology, agriculture, and forestry shall have advanced far enough to require quantitative information on the dynamic aspect of soil aeration.

BACTERIAL SPOT OF LIMA BEAN[1]

By W. B. Tisdale, *Assistant Plant Pathologist, Florida Agricultural Experiment Station*, and Maude Miller Williamson, *formerly Instructor in Plant Pathology, University of Wisconsin*[2]

INTRODUCTION

During recent years lima bean plants in the vicinity of Madison, Wis., have been affected with a spot disease which was recognized as distinctly different from the blight caused by *Bacterium phaseoli* E. F. S. This disease was first noted in 1917 in several home gardens at Racine, Wis. In August of that year, the disease was also observed to be quite serious in some of the gardens at Madison. Scarcely a plant could be found free from the spotting and some of the plants were affected so badly that most of the blossoms and small pods were shedding. During the autumn of 1917 intensive investigations were begun which were continued during the winter. After this no further observations were made until the summer of 1919. During 1919 the spot appeared on the first leaves, but the plants outgrew it and remained practically free from it throughout the summer. It appeared again in the spring of 1920 and continued to develop throughout the summer of that year but not so seriously as in 1917.

In June, 1921, the senior writer visited several fields of lima beans on Long Island but was unable to find any signs of the spot disease. The plants at this time, however, had only the first two leaves present, and the weather had been dry and hot since the seed was planted. In September of this year both the spot disease and blight were prevalent and, in some cases, serious in the gardens at Madison.

LITERATURE

The literature up to the present time seems to give no description of the disease or of its causal organism, although a bacterial disease of lima beans having at least some characters in common with the spot disease has been mentioned.

The blight attributed to *Bacterium phaseoli* E. F. S. has been known for several years (*7*)[3] to attack lima bean. Halstead (*4*), in 1892, reported the occurrence of a bacterial disease of both common beans and lima beans on the farm of a western seed company and stated that the disease had been known on that farm since 1886. Beach (*1*) described a bacterial disease of lima beans in New York which he suggested as being distinct from the one caused by *B. phaseoli*. Beach's description of the disease is in part as follows:

> So far as noticed, these spots are never black, but often have a reddish-purple border inclosing an area of light red color. The spots gradually increase in size and develop a straw colored center of dead tissue.

[1] Accepted for publication May 2, 1923.

[2] The writers wish to make grateful acknowledgment to Prof. L. R. Jones, of the University of Wisconsin, for helpful suggestions in the final preparation of the manuscript.

[3] Reference is made by number (italic) to "Literature cited," p. 153.

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This description characterizes the disease under consideration in this paper more nearly than it does the blight caused by *B. phaseoli*, as the two diseases have been seen at Madison, Wis. In 1898, Sturgis (*8*) described a bacterial disease of lima beans in Connecticut which he attributed to *B. phaseoli* E. F. S. However, his description of the disease more nearly characterizes the spot disease than the blight. Manns (*5*) has also described a bacterial organism which is pathogenic upon certain legumes, including lima beans. He did not describe or illustrate the disease of lima beans, but his report upon cultural and morphological characters precludes the possibility of the two organisms being the same.

SYMPTOMS

Since the bacterial blight caused by *B. phaseoli* also attacks the lima bean, it is well to differentiate between this and the bacterial spot. The spots of the two diseases appear quite different from the beginning and can be easily distinguished even when the two occur on the same leaf or pod as they frequently do.

BACTERIAL BLIGHT

The blight, caused by *B. phaseoli* E. F. S., attacks all the parts of the lima bean plant which are subject to the spot disease. The lesions on all parts of the plant are very similar to those produced on similar parts of the common bean (*Phaseolus vulgaris*) by *B. phaseoli*. Young lesions on the leaves are water-soaked, and the tissues surrounding them soon dry out, causing large blighted areas which generally extend to the leaf margin. Exudate appears on the lower surface of the young lesions and dries out, forming a thin film or scale. The blighted areas are not as brown in color as the bacterial spot (Pl. 1, A), but the small veins near the spots are often red. On the pods and stems the young lesions are also water-soaked and accompanied by films or crusts of yellow exudate. The lesions extend through the walls of the pod and attack the seed. A thick yellow exudate is often produced both outside of and beneath the seed coat.

BACTERIAL SPOT

On the leaves where this disease is very conspicuous, it is characterized by brown to purplish-colored lesions, which vary from somewhat irregular to almost circular shapes (Pl. 1, B). The spots are never at any time water-soaked. At first they appear as small brown points on the upper surface and enlarge very rapidly for the first few days. On the lower surface the young spots are depressed and the margins are a lighter color than on the upper surface. As the spots enlarge, the centers dry out and turn gray or straw colored and the margins remain a glistening, purplish red color. Single spots are usually from 1 to 3 mm. in diameter and may be quite generally scattered over the leaf surface, although they are occasionally grouped and confluent, especially on the first leaves. When spots thus coalesce, lesions several millimeters in diameter are formed. In the latter case the centers of the lesions break out and give the leaves a ragged appearance. The lesions are usually smaller and more regular in shape on the upper leaves. On the King of the Garden variety the spots cause a curling or distortion of the young leaves. The disease also occurs on the veins and petioles where it appears as characteristic reddish-brown or glistening caramel-colored streaks. The streak usually occurs

on the upper or grooved side of the petiole, and may extend throughout its length. When it attacks the base of the leaf stalks, the leaves shed prematurely.

The disease is not confined to the leaves but also occurs in the stems and pods. On the stem the lesions vary in size from 1 mm. to several centimeters long (Pl. 2). The lesions on the stems as well as on the petioles extend into the vascular tissue, but no evidence was found to indicate that the organism progressed very far through the vessels. A straw or light wine-colored exudate appears on the stem lesions in the moist chamber and occasionally in the field, which dries down and forms a thin glistening crust. In several cases the peduncles were found attacked and even completely girdled. In such cases, and when the pedicels are attacked, the blossoms and young pods shed. Brown spots have been observed on the blossoms of diseased plants, but the organism has never been isolated from them.

On the pods the disease begins as small brown spots surrounded by a water-soaked halo. The lesions may occur both on the side of the pod, and also along the sutures where they become streaks (Pl. 3, A). They may extend through the walls of the pod and attack the seed, in which case the veins of the seed coat around the invaded area often exhibit a reddish color and occasionally a white sticky exudate underneath the seed coat. In some cases spongy excrescences were found protruding from the inner walls of the pod beneath the surface lesions. A crust of exudate was found on some of the pod lesions in the field. When diseased pods were kept over night in a moist chamber, drops of a straw-colored exudate appeared on the lesions (Pl. 3, B). These drops later dried down to form a thin crust.

Isolations made from stems, leaves, and pods, such as here described, have produced typical lesions when applied to healthy plants and reisolations have yielded the typical organism.

SEASONAL OCCURRENCE

Since the bacterial spot of lima bean has been under observation a variety of conditions has been found to exist. In 1917 the disease appeared on the first leaves and progressed steadily throughout the season until frost killed the plants. In 1918 no observations were made. Again in 1919 the disease appeared early in the season, but its development was checked in July with the onset of dry weather and the subsequent growth of the plants was free from the disease. On the other hand, the blight caused by *B. phaseoli* was common in lima beans at Madison, especially in the phytopathological garden where the lima beans were planted beside common beans. Practically the same conditions obtained in 1920. In 1921 the disease did not appear until later in the summer, but once started, it continued to develop until frost. By this time the plants were very ragged. It thus appears that frequent rains and favorable temperature are necessary for dissemination of the organism and development of the disease.

ECONOMIC IMPORTANCE

Under field conditions as observed in 1917 bacterial spot is an important disease of the foliage. Practically no pods were set in the phytopathological garden after August 15 because of the severe infec-

tion of leaves and pedicels. When free from disease, the plants continue to grow and set pods until frost, which in the vicinity of Madison, Wis., is usually not earlier than September 10. Such an outbreak in commercial fields would cause a considerable reduction in yield. Aside from this epidemic, however, the disease has been of little practical importance except late in the season of 1921, when it caused heavy infection of the leaves.

Except for the probability of the organism being carried over on the infected seed, nothing is known of the method of overwintering. In Wisconsin, even though the young seedlings become infected, it appears that subsequent weather conditions are, as a rule, unfavorable for further development of the disease. With such sporadic outbreaks the disease will probably never be of any great economic importance under Wisconsin conditions. In States where the lima bean is grown commercially the disease should be considered a factor of economic importance, provided that the climatic conditions are favorable for its development.

THE ORGANISM

ISOLATION

Microscopic observations of sections of young lesions show the invaded tissue to be swarming with bacteria. The organism was isolated from the leaf tissue in practically pure culture by dipping the diseased tissue in 95 per cent alcohol for an instant, immersing in mercuric bichlorid (1 to 1,000) for one minute, rinsing through three or four sterile water blanks, and crushing in a tube of beef broth. After one-half to one hour dilution plates were poured from the tube of macerated tissue.

In the isolations from stems and pods a wet-shining, rapidly-growing, yellowish organism almost invariably appeared on the plates along with the white organism, but several inoculation experiments proved that it was not pathogenic. Pure cultures of the pathogenic organism have been obtained by touching a drop of the exudate on pods with a sterile needle and transferring directly to agar slopes.

During the course of investigations a number of strains of the organism have been isolated each year and successful inoculations made with them. A comparison of cultural characters showed that all were quite similar. The first strain isolated, designated 1a, has been studied most intensively and is presented as the type strain.

MORPHOLOGY

The organism is a short rod with rounded ends, usually occurring singly or in pairs in young cultures. Short chains have been observed in old agar cultures and in beef-peptone broth containing 4 per cent sodium chlorid. When stained from 3-day-old beef-peptone agar cultures with gentian violet or Loeffler's methylene blue, the cells measure from 0.3 to 0.7 μ in diameter and from 0.7 to 2.2 μ in length, averaging 0.5 by 1.5 μ.

Both Caesar-Gill's and Duckwall's modification of the Pitfield flagellum stains have shown the organism to be motile by one to several polar flagella (Pl. 3, C). No endospores or involution forms have been observed. Capsules were not demonstrated by Welch's staining method from potato agar, nutrient agar, or nutrient broth cultures. The organism is gram negative and nonacid fast.

CULTURAL CHARACTERS

Unless otherwise specified, all cultures were incubated in the dark at about 25° C., a temperature very favorable for the organism. Color notations have been made in comparison with Ridgway's Color Standards.[4] In most cases acidity of the medium was determined by both Fuller's scale and hydrogen-ion concentration. Phenolphthalein was used as an indicator in the determinations by the Fuller's scale method. The different agar media were made according to E. F. Smith's formulae (*6*) except that 1.8 per cent of bacto agar was used.

AGAR POURED PLATES

On + 10 potato agar colonies appeared in about 24 hours, and at the end of 4 days were about 4 to 5 mm. in diameter. They were creamy white, glistening, smooth, circular, entire, umbonate, and opaque. Old cultures become a pale olive-buff color. The surface was usually rugose, but may appear more or less smooth. The consistency was butyrous. Buried colonies were lenticular.

On + 10 nutrient agar colonies appeared in about 48 hours, and after 4 days were 3 to 4 mm. in diameter. They were flat, circular, smooth, glistening, butyrous, opalescent, with faint gyrose to marmorated markings. The margins were undulate. After 3 to 4 days the medium began to turn yellowish-green underneath the colonies, and the color gradually diffused out into the medium. Buried colonies were lenticular.

AGAR STABS

Stabs in + 10 potato agar showed abundant surface growth, rather convex, glistening, and creamy white. After several days the growth may spread over three-fourths to almost the entire surface. Scanty growth developed along the line of stab for a short distance. There was no change in the medium.

Stabs in + 10 nutrient agar developed slightly less abundant growth than on potato agar and was flat, opalescent, and glistening. There was scant growth along line of stab for a short distance. The medium was greened.

AGAR STROKES

On + 10 potato agar the slant stroke cultures made an abundant, flat, echinulate, smooth, glistening, butyrous, opaque growth. The color was creamy white when young, but when 10 days old it was pale olive-buff by reflected light. The medium was unchanged.

On + 10 nutrient agar slants growth was slightly slower and less abundant than on potato agar. It was thinner and fluorescent. The surface was smooth and glistening. It was echinulate, translucent, and butyrous. A slight putrefactive odor was produced. The medium was greened, and after about 10 days, the growth was about the same color as the medium.

BLOOD-SERUM AGAR

Stroke cultures on blood-serum agar gave abundant growth. The medium was browned and slowly liquefied. In small tubes containing about 5 cc. of the medium and kept at about 25° C. liquefaction was complete in 4 weeks.

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[4] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C. 1912.

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STARCH AGAR

There was no evidence of diastatic action on potato starch suspended in beef-peptone agar when tested with iodin.

GELATIN PLATES

Colonies appeared in about 36 hours and grew fairly rapidly. They were circular with entire margin. Liquefaction began shortly after the colonies appeared and in 3 days had produced saucer-shaped depressions 6 to 7 mm. in diameter.

GELATIN STABS

Surface growth was abundant in 3 days. There was no growth in lower part of stab. Liquefaction began in about 3 days and was complete in 60 days in tubes containing 10 cc. of medium. It was crateriform at first, but later became stratiform. In tubes uniformly inoculated and incubated at 20° C. for 7 days, liquefaction advanced 10 mm.; in 10 days, 15 mm.; and in 30 days, 27 mm. The liquefied medium was fluorescent, greenish, with heavy surface growth which when agitated, came down in flocculent masses, thus forming a heavy white precipitate.

NUTRIENT BROTH

In +10 beef-peptone broth a thin fringed pellicle formed and dropped as a unit after 3 or 4 days. Uniform clouding began in 12 to 24 hours and became heavy and persistent within 3 to 4 days. A putrefactive odor was produced. Abundant precipitate was formed which was viscid on agitation. The medium turned yellowish green after 2 days and was alkaline to litmus. No hydrogen sulphid or ammonia was produced. Erlich's reagent showed a positive reaction for indol after 4 days.

POTATO CYLINDERS

Growth on steamed potato cylinders was pale yellow in color, flat, spreading, and viscid. After 7 to 10 days the cylinders became a vinaceous buff color.

MILK

In plain milk there was no apparent coagulation, but a slight creamy consistency developed after 3 to 4 days. Peptonization began at the top after 2 days and was complete at the end of 12 to 14 days. A heavy white, granular precipitate developed. After digestion the medium was honey-yellow in color, of thin gelatinous consistency, and strongly alkaline.

METHYLENE BLUE MILK

The blue color was reduced in 2 or 3 days, but a pale olivine color appeared in the cleared, digested part after about 5 or 6 days. After about 1 month the medium became honey-yellow and viscous.

LITMUS MILK

Alkaline reaction appeared before beginning of protein digestion and persisted until the litmus was reduced.

COHN'S SOLUTION

In Cohn's solution there was no growth.

FERMI'S SOLUTION

A thin fimbriate pellicle was formed and sank to form a flocculent precipitate. Heavy uniform clouding occurred within 3 to 4 days. The medium became milky fluorescent within the first 3 or 4 days and later became a bluish green color by reflected light.

USCHINSKY'S SOLUTION

The action was similar to that in Fermi's solution except the pellicle was more viscid and the bluish color was less prominent.

LITMUS AGAR WITH SUGARS

Dextrose, maltose, and lactose, respectively, were sterilized in concentrated water solution and added to sterilized litmus agar in sufficient quantities to make a 1 per cent sugar solution. Five cc. of a saturated water solution of azolitmin was added to each liter of the neutral nutrient agar.

Litmus lactose and litmus maltose agar slopes produced good growth. A very faint reduction of litmus occurred at the bottom of the slope after 2 weeks, but the normal color soon reappeared and continued exactly like the controls throughout the duration of the experiment, about 3 months.

On litmus-dextrose agar slopes there was abundant growth and distinct acid reaction. In 3 days the medium began to redden underneath the growth at the base of the slope and this coloration progressed for some depth into the medium. Reduction began underneath the heaviest growth and was complete within 2 weeks. The medium was then an amber or reddish brown color. After about 17 days the litmus color completely reappeared and remained about the same color as the controls.

DUNHAM'S SOLUTION

Moderate uniform clouding developed in 3 days. No ring or pellicle developed. A flaky precipitate formed which broke up when agitated.

RELATION TO OXYGEN

The organism is aerobic. No growth developed in agar in Roux tubes. Tubes of nutrient agar were also inoculated while melted, and rolled to distribute the organism throughout. No growth developed at a depth greater than 1 mm. below the surface. No clouding developed in the closed arm of fermentation tubes with any of the sugars tested.

FERMENTATION TUBES

The tests were made in 2 per cent peptone solution (+12 Fuller's scale, P_H. 8.2 to 8.4) with 2 per cent, respectively, of each of the following carbon compounds: glycerine, mannit, dextrose, lactose, maltose, and saccharose.

The sugars and broth were sterilized separately at 12 pounds pressure and the desired quantities mixed in the culture tubes and flasks under aseptic conditions. Eight tubes were used for each sugar. Clouding began after 24 hours. At the end of 6 days heavy clouding was noted in the open end of all tubes and a precipitate later developed. In all cases there was a definite line of demarcation across the inner part of the U

and there was no visible growth in the closed arm. With dextrose the line of demarcation was only slightly above the inner part of the U. No gas formed in the closed arm in any case.

ACID PRODUCTION

In addition to the fermentation tubes used for determining gas production, three 100 cc. Erlenmeyer flasks were prepared in triplicate for each of the sugar solutions.. Fifty cc. of the peptone-sugar solution was added under aseptic conditions to each flask. The media were then uniformly inoculated and incubated at 25° C. The acidity was determined by both Fuller's scale and hydrogen-ion concentration methods after different periods of incubation. Twenty cc. of the solution was removed with a sterilized pipette from each flask for the determinations. Of these cultures acid was produced only in those containing dextrose and saccharose. The other cultures became slightly more alkaline as indicated in Table I.

Table I.—*Production of acid from sugars and glycerine*

Carbon compounds used in experiment.	Control.		Reaction after different periods of incubation.			
			10 days.		30 days.	
	Fuller's scale.	P_H.	Fuller's scale.	P_H.	Fuller's scale.	P_H.
Dextrose	+12	8.0	+14	7.6	+48	4.4
Saccharose	+13	8.2	+22	6.4	+45	4.6
Mannit	+12	8.0	+10	8.2	+16	8.6
Maltose	+13	8.2	....	....	+11	8.6
Lactose	+13	8.2	+12	8.4	+10	9.2
Glycerine	+13	8.2	+11	8.6	+11	8.6

NITRATE BROTH

In fermentation tubes nitrate broth gave heavy clouding in the open end and none in the closed end; no gas was formed. A positive test for ammonia was obtained with Nessler's reagent at the end of 2 and 3 weeks. Trommsdorf's reagent gave a negative test for nitrites. Therefore nitrate was reduced completely to ammonia.

DIGESTION OF CASEIN

Clear casein agar plates were inoculated with the type organism. After 3 days a test with 1 per cent hydrochloric acid indicated that the casein was digested around the colonies. After 2 weeks the whole plate was clear, indicating rapid digestion.

VITALITY ON CULTURE MEDIA

Cultures kept in the ice box may be kept alive indefinitely on nutrient agar by transferring every month. The organism may be recovered from 2 to 3 months old cultures on potato or nutrient agar by transferring to nutrient broth. At laboratory temperature potato and nutrient agar cultures lose their viability after 2 or 3 months.

OPTIMUM REACTION AND TOLERATION LIMITS

Beef-peptone bouillon was adjusted to each of the following reactions with sodium hydroxid and hydrochloric acid: +32, +25, +22, +20, +15, +10, +5, +2, 0, −5, −7, −12, and −16. These were uniformly inoculated with 48-hour-old broth cultures and incubated at 24° C. At the end of 1 week there was growth in all tubes between +25 and −7. Heavier clouding developed at +20 than at +10, but the greenish color did not appear in the +20 medium. A heavy precipitate developed at 0, +2, +5, and +10. At −5 there was only moderate clouding, and very slight growth at −7. The optimum temperature for growth is, therefore, +10 to +20 Fuller's scale.

TOLERATION OF SODIUM CHLORID

Beef-peptone bouillon titrating +15 (Fuller's scale) and containing 0.25, 0.5, 1, 1.5, 2, 3, 4, and 5 per cent, respectively, of sodium chlorid was uniformly inoculated from +10, beef-peptone agar. There was heavy clouding in 0.25, 0.5 and 1 per cent after 2 days, and a thin pellicle was formed. The pellicle would come down intact upon agitation. There was only slight clouding and a small quantity of ropy precipitate in 3 and 4 per cent after 2 days. No clouding was apparent in 5 per cent solution. After 8 days the heaviest clouding was manifest in 0.5 and 1 per cent. There was a pellicle and an amorphous precipitate. There was a ropy precipitate and medium to light clouding in all concentrations except 5 per cent. No clouding occurred in 5 per cent solution, but a small amount of ropy precipitate developed.

Temperature Relations

In +10 nutrient broth the thermal death point lies between 49° and 50° C. This was determined by inoculating 5 cc. portions of the broth in thin-walled test tubes of 13 mm. diameter, by means of 2 loops of a 48-hour-old broth culture and by allowing them to incubate one-half hour before plunging them into water held at the desired temperature. Five inoculated tubes were held for 10 minutes at each temperature in two different tests and were then plunged immediately into cold water until they were thoroughly cooled. After this they were incubated at 25° C. Good growth took place in all tubes heated up to and including 49° C., but none occurred above that.

The relative optimum temperature was determined by incubating 3 inoculated tubes of nutrient broth and potato agar slants at temperatures ranging from 3 to 39° C. at intervals of two or three degrees. This range was repeated, and a third series was run at the higher temperatures 25°, 30°, 31°, 33°, 34°, 37°, 38° C., respectively. Results were uniform in all three series. After 3 days the optimum growth occurred at 28° to 30° C. in broth, and at 26° to 30° C. on agar. As the period of incubation increased, the maximum growth on agar dropped slowly, appearing at 24–26° C. after 18 days. Below 23° C. the clouding in broth was not so strong and the fluorescence was much more marked than at higher temperatures. Growth was very slow at 3° C., but cultures remained viable for some time at that temperature. At 35° C. there was good growth after the first 24 hours but the increase was slow thereafter and the characteristic color did not develop. Very slight growth occurred above 35° C. After being incubated at 37 to 38° C. for 1 week on potato agar, the organism was killed.

Desiccation

The organism as it occurs in the host tissues seems to be very resistant to drying. Successful isolations were made from diseased leaves which had been kept in the herbarium 2½ years. On potato agar cultures kept in the ice box, where growth is abundant, the organism was viable after 3 months. When beef-peptone bouillon was inoculated with transfers from these cultures, good growth developed. The organism is very readily killed when dried on sterilized cover glasses. Smears were made from 2-day-old broth cultures on sterilized cover glasses and were placed in sterilized Petri dishes. All cells were dead at the end of 24 hours.

TECHNICAL DESCRIPTION

On the basis of the foregoing studies, the organism is characterized briefly as follows:

Bacterium viridifaciens n. sp.[5]

Cylindrical rods rounded at ends, solitary or occasionally in pairs, in short chains in old cultures; individual rods 0.3 to 0.7 by 0.7 to 2.2μ; motile by one to several flagella; aerobic; no spores; no capsules in agar or beef broth cultures.

Superficial colonies on nutrient agar, circular, smooth, glistening, flat, butyrous-opalescent, with faint gyrose to marmorated markings; margin undulate; medium stained a pale lumiere green.

Gelatin moderately liquefied; no acid produced in milk; casein digested without coagulation; litmus reduced in milk; hydrogen-sulphid gas not produced; nitrates reduced; acid produced in media containing dextrose and saccharose; no growth in Cohn's solution; thermal death point between 49° and 50° C; not acid fast; gram-negative.

Group number 211.2322133.

Pathogenic on varieties of *Phaseolus lunatus* Linn, forming lesions on leaves, stems, and pods.

Type locality: Racine, Wis.

Distribution: Eastern and southern Wisconsin.

INOCULATION EXPERIMENTS

The bacterial spot has been reproduced many times with characteristic symptoms under greenhouse and field conditions by spraying water suspensions of the organism on healthy plants. From lesions produced in this way the original type organism has been repeatedly recovered. The disease could always be produced with a newly isolated strain of the organism by spraying a water suspension of the organism upon uninjured leaves, both old and young ones, and placing the plants in a moist chamber from 12 to 24 hours. Wounds are unnecessary for infection. When carried in culture for several months, the organism became less pathogenic.

Several varieties of lima beans were used in the inoculation experiments including Fordhook, King of the Garden, Dreer's Bush, Burpee's Bush, and Henderson's Bush. All varieties were susceptible, but the spots developed to larger size on the Fordhook than on the other varieties.

Besides these varieties of lima beans, Alaska peas and wax beans were inoculated under both greenhouse and field conditions. No infection developed on any of the plants.

In making the inoculations in the greenhouse the following method was usually employed: Lima beans were planted in previously sterilized

[5] According to Migula's classification and Buchanan's revision (2), the combination would be *Pseudomonas viridifaciens* n. sp.

soil, in 8-inch pots and when the first two or more leaves had developed they were inoculated with an atomizer spray of a water suspension of a 5 to 10-day old culture of the organism. The plants were then placed in a warm, damp chamber for 24 hours, after which they were removed to a greenhouse bench where the temperature was about 22° C.

Practically the same method of inoculation was used in the field. Plants free from the disease were selected, inoculated with atomizer spray, and then covered with a glass moist chamber over night or in humid cloudy weather they were left uncovered. In all these experiments the plants were inoculated before the blossoms began to appear.

Both inside and out of doors typical lesions began to develop in from 2 to 7 days after the plants were inoculated.

Control plants were always sprayed with sterile water and incubated under the same conditions as inoculated plants, but in no case did the disease develop in them.

RELATIONS TO HOST TISSUE

Inoculation experiments have shown that wounds are unnecessary for infection. Razor sections of recently infected leaf tissues showed invasion to be in the parenchyma. Young lesions in leaves were fixed in Gilson's solution, imbedded in paraffin, sectioned, and stained with Ziehl's carbol-fuchsin. A study of diseased material prepared in this way showed that the organism enters through the stomata and works its way into the underlying tissue between the cells. The middle lamella was destroyed and the cells were crowded apart by the increasing numbers of bacteria. In the later stages the walls may be torn or collapse and the bacteria invade the cell cavities. The infected areas dry out and become sunken on the lower surface. No vascular invasion was observed.

OVERWINTERING AND CONTROL

Up to this time practically no experimental work has been done in relation to the overwintering of the bacterial spot organism and the methods for controlling the disease. It has been noted, however, that the disease attacks the pod and seed which suggests the possibility of the organism living overwinter with the seed. In 1917, when severe pod infection developed, only a few infected pods matured. These were picked, and the seed was planted the following spring in the greenhouse, but no infection appeared on any of the plants.

It should not be inferred from this one experiment, however, that diseased seed is not a source of primary infection. Since a pathogenic strain of the organism has been isolated from diseased leaves kept for two years in a herbarium, it seems quite possible for the organism to live over winter in the diseased seed as well, and possibly in the diseased leaves in the field. It has been noted also that the disease appeared year after year in the same garden in the first young leaves, but rarely became serious until later in the summer when both old and young leaves were attacked. This condition is not universal, since gardens have been found free from the disease early in the season and several gardens under our observation have remained free from it throughout the entire year.

Until further work on overwintering and dissemination has been done, it seems inadvisable to recommend any specific control measures. Since the causal organism of bacterial spot attacks the seed, seed disinfection

at once suggests itself. However, many of the seed lima beans purchased on the market have cracked seed coats and will not withstand treatment with mercuric bichlorid or formaldehyde solutions of sufficient concentration and duration to kill the bacteria harbored in the tissues. Seeds with unbroken testa will withstand only a mild treatment with water solutions without injury because the testa wrinkles and breaks when wet. Since only a small quantity of seed known to be infected was available, no tests were made to determine an effective method of seed treatment. Observations made in small gardens and inoculation experiments have shown no indication of resistant varieties. These observations, however, have been made on too small a scale to draw any definite conclusions. If the disease should become serious in sections where lima beans are grown on a commercial scale, the development of resistant varieties would seem to be the most feasible means of control.

SUMMARY

(1) The spot of lima bean described in this paper has been observed in the home gardens in the southeastern part of Wisconsin for several years. So far as known, it has not been reported from other sections of the United States.

(2) All varieties of lima beans so far tested are susceptible. No other species of legumes have been infected.

(3) The disease is characterized on the leaves, where it is most conspicuous, by more or less irregular spots with grayish center and definitely delimited purplish borders. In early stages the disease is characterized by purplish or brown spots, slightly depressed on the lower surface. In late stages the center becomes gray. The spots are never water-soaked and no exudate appears.

(4) Petiole, stem, and pod lesions accompany the disease on the leaves. A small amount of exudation has been observed on the pods in the field. When kept in a moist chamber straw-colored exudate appeared on the pods in large droplets.

(5) The disease was severe in its attack on lima beans in the vicinity of Madison, Wis., during the season of 1917. Such attacks occurred under conditions of heavy rainfall and moderately high temperature. During seasons with high temperature and light rainfall the disease was of little importance. While the disease may never be of great economic importance in home gardens, it has the potentialities, under favorable weather conditions, of causing considerable reduction in yield and would necessitate remedial measures where lima beans are grown commercially.

(6) The causal organism is a medium rod motile by one to several flagella which is described as *Bacterium viridifaciens* n. sp. It grows readily on a variety of culture media, producing white, glistening, opaque colonies. Beef extract agar, beef broth, Uschinsyk's and Fermi's solution are turned green. It produces acid without gas in media containing dextrose and saccharose, reduces nitrates, is non-acid fast and gram-negative. It is highly resistant to desiccation in diseased leaves but is killed within 24 hours on sterile cover glasses. There seems to be a gradual loss of pathogenicity when it is grown in artificial culture.

(7) Foliage and stem infection is readily obtained by spraying water suspensions of young cultures on healthy uninjured plants. No pod inoculations have been made.

(8) Since the seed is attacked, it seems very probable that the organism may live over winter on the seed. It is also possible that the organism may live over winter on the bean refuse, as it is known to live more than two years in herbarium material.

(9) Control measures have not been worked out. Because of the organism passing through the seed coat it seems impossible to kill the organism with watery solutions of disinfectants without injuring the seed. In view of this fact, it seems better to rely upon the selection of disease-free seed and development of resistant varieties in localities where lima beans are grown commercially.

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PLATE 1

A.—A comparison of bacterial spot (left) with bacterial blight (right) in King of the Garden lima bean. The bacterial spot is the result of artificial inoculation with water suspension of *Bact. viridifaciens*. The bacterial blight shows natural infection as it appeared in the gardens in the vicinity of Madison, Wis. About natural size.

B.—Bacterial spot in King of the Garden lima bean. A result of artificial inoculation of plants grown in the greenhouse. Photographed two weeks after inoculation. About natural size.

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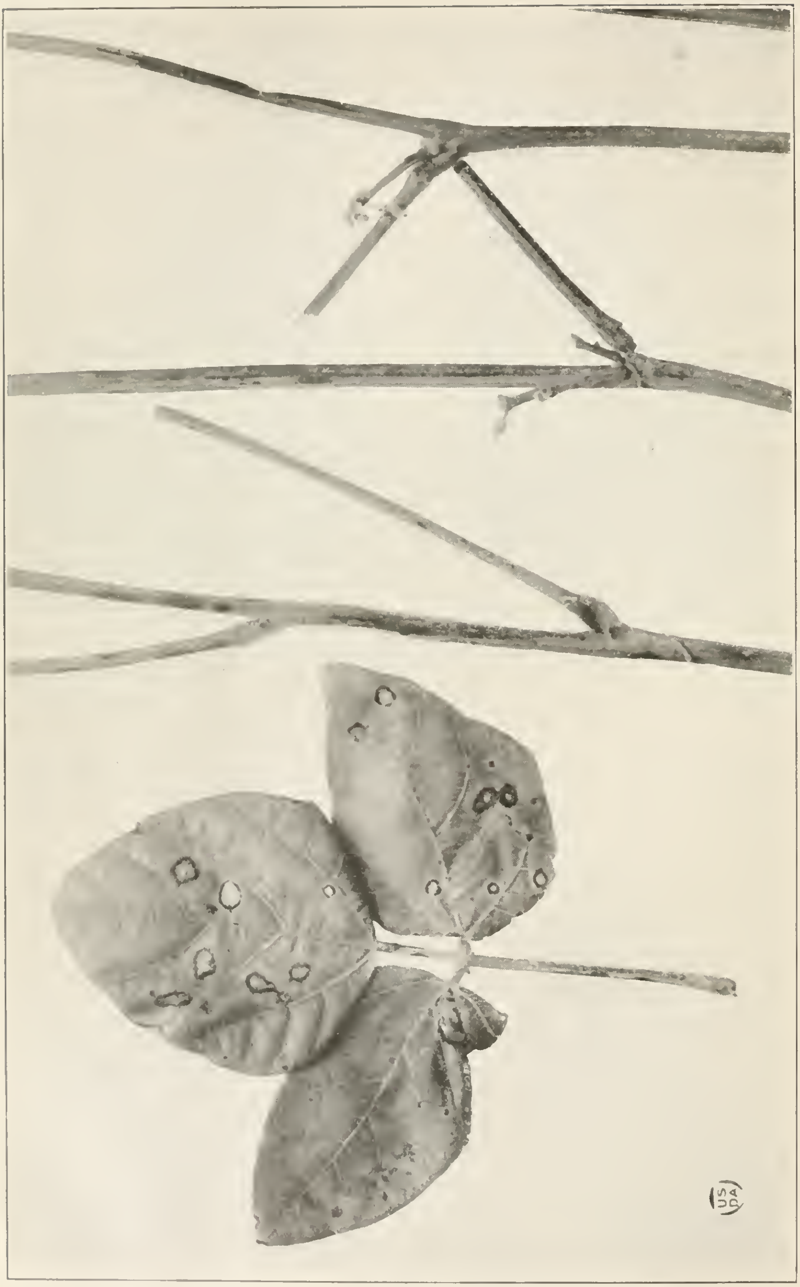

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PLATE 2

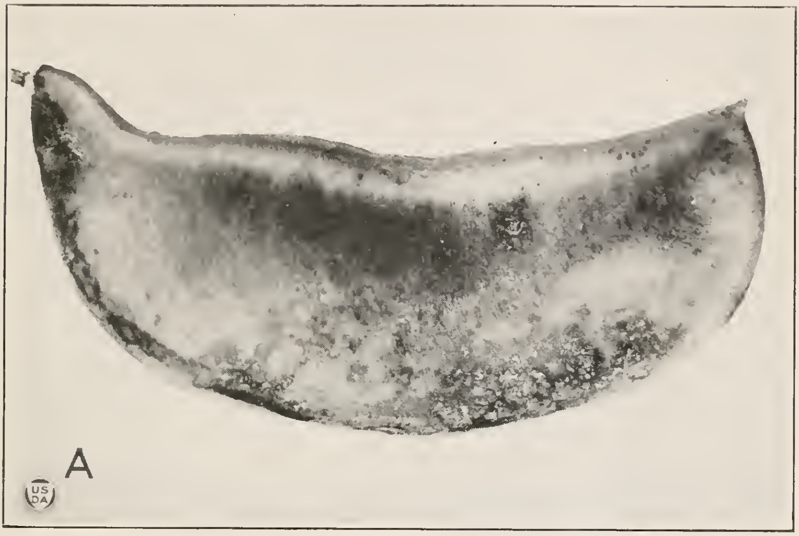
Bacterial spot on leaf and stems of lima bean. Natural infection, showing the characteristic dark borders of the leaf spots and the dark streaks on the stems. About natural size.

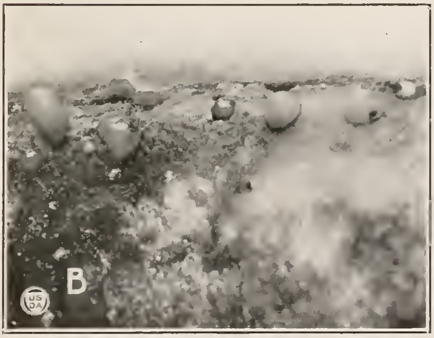
PLATE 3

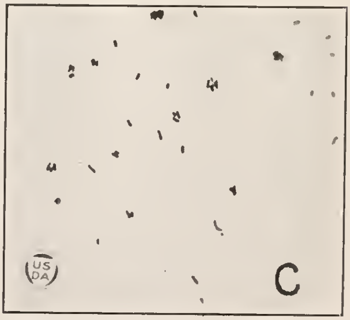
A.—Bacterial spot on pod of lima bean. Natural infection, showing dark-colored lesion extending along the dorsal suture. X 2½.

B.—A portion of lima bean pod affected with bacterial spot. Natural infection, showing drops of exudate which came out over night in a moist chamber at room temperature. X 5.

C.—Photomicrograph of *Bacterium viridifaciens* from a 24-hour old nutrient agar culture, and stained with the Duckwall modification of the Pitfield method to show flagella. Note that two cells are undergoing the process of division. X 850.







HYDROGEN-ION CHANGES INDUCED BY SPECIES OF RHIZOPUS AND BY BOTRYTIS CINEREA[1]

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Investigations with certain species of Rhizopus grown on nutrient solutions have suggested that some of them increase the hydrogen-ion concentration, while other closely related species decrease or have no action on the acidity. These results, obtained more or less incidentally, suggested that no sweeping generalizations could be drawn for all fungi from the results obtained from a few. In fact, they suggested that the results obtained from the study of one or more species could not be applied to all species of a single genus. It has been demonstrated by different investigations that *Sterigmatocystis niger* (*12*),[2] *Aspergillus niger* (*3*), *Penicillium glaucum*, and *Botrytis cinerea* (*9*), *Citromyces pfefferianus*, *C. glaber* (*6*), and others produce acids (not the same acid in all cases) when grown in artificial culture media. Matsumoto (*11*) in a physiological study of 15 different isolations of Rhizoctonia found that the general tendency of these organisms was to increase the actual acidity during growth, the increase seemingly being proportional to the increase in growth.

In investigations carried out by the writers it was shown that when *Rhizopus tritici* Saito was grown on sweet-potato decoction, a vigorous cell-wall-splitting enzym was produced, which separated the cells of sweet-potato disks along the line of the middle lamellae so that coherence was entirely lost. It was found, however, that when a modified Czapek's nutrient solution with glucose as a source of carbon was employed as a substrate, the enzym was not produced but that a certain amount of maceration of the tissue of raw disks resulted, which was found to be caused by the acid formed. The hydrogen-ion concentration was about P_H 1.70 to 1.80. If, on the other hand, pectin obtained from the carrot was substituted for glucose in the substratum, the cell-splitting enzym was secreted and the highest hydrogen-ion concentration was about P_H 3.5. This is some increase in acidity over that of the original solution, but the total acidity was not sufficient to dissolve the middle lamellae.

The writers (*4*) found that the different species of Rhizopus varied considerably in the amount of pectinase produced under identical conditions. They also found that some of the species whose enzym acted feebly upon raw disks, decayed sweet potatoes, under natural conditions, quite as vigorously as those which macerated tissue rapidly. With these facts in mind it was suspected that some relationship might exist between the ability of the different species to produce acids and their capacity for decaying sweet potatoes under natural conditions. It was shown that *Rhizopus nigricans* Ehrnb. did not decay sweet potatoes as rapidly as *R. tritici*; also that the action of the enzym in the solution in artificial culture on which it grew was very much slower than that of the latter organism. It was thought that this difference between some of

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[1] Accepted for publication May 2, 1923.
[2] Reference is made by number (italic) to "Literature cited," p. 163–164.

the species might be due to the difference in the amount of acid produced, which acted alone in the absence of pectinase or as a co-enzym. It was accordingly proposed to make a comparison of the changes in hydrogen-ion concentration produced by the different species. A study of the influence of light on some of the vital activities of *R. tritici* was also made.

COMPARISON OF DIFFERENT SPECIES OF RHIZOPUS

A comparison of the different species of Rhizopus growing on sweet-potato decoction was made. A sufficient quantity of the decoction was prepared at the beginning to carry out the entire investigation. Every precaution was taken to grow all the species under identical conditions. A number of flasks were prepared for each species and several held as controls. The inoculated flasks and controls were incubated at a temperature of from 23° to 24° C. in the dark. At the end of the growth period (7 days) the substrate was filtered through cotton to separate it from the mycelium. The solution on which each species had grown was collected into one sample and after thoroughly mixing, its hydrogen-ion concentration was tested. The control solution (collection from several inoculated flasks) was found to have a P_H of 5.35.

TABLE I.—*Showing the hydrogen-ion concentration of the solutions on which 11 species of Rhizopus had grown for 7 days*

Species.	Artocarpi.	Nigricans.	Microsporus.	Reflexus.	Tritici.	Delemar.	Oryzae.	Nodosus.	Arrhizus.	Maydis.	Chinensis.
P_H.	4.00	5.44	5.62	3.42	3.07	3.07	3.07	3.07	3.24	3.22	4.31

Table I shows some interesting data. The sweet-potato decoction did not reach as high a hydrogen-ion concentration as is usually obtained in Czapek's nutrient solution. It will be seen that two species, *R. nigricans* and *R. microsporus* van Tieghem, make the solution less acid, while in all other cases the acidity of the solution is increased. A number of the fungi have changed the P_H value to a little above three. Two species, *artocarpi* Racib. and *chinensis* Saito, have brought the P_H of the solution to 4.0 and 4.31, respectively. It is interesting to note the exact similarity in the results obtained with *tritici*, *delemar* (Boid.) Wehmer and Hanzawa, *oryzae* Went and Pr. Geerlings, and *nodosus* Namysl. These four species are very similar morphologically, and in many cases it is not possible to distinguish between them. The writers have several times called attention to the fact that these four species fall into one group and may eventually be found to be identical taxonomically.

ACIDITY IN RELATION TO MACERATION

In a previous publication (*4*) it was shown that the time required to macerate raw sweet potato disks by the enzym secreted by *Rhizopus nigricans* in the solution on which it grew and that retained by the mycelium, was much longer than that required by such species as *tritici*, *delemar*, and others. However, *Rhizopus nigricans* is the species responsible for most of the decay of sweet potatoes in storage. It does not, however, decay potatoes as rapidly as some of the other species. Thus far the writers have been unable to offer any explanation of why this species will readily decay sweet potatoes, causing a loss of coherence of the cells in an identical manner, but producing a much less amount of pectinase in artificial cultures. In attempting to offer an explanation of

this interesting phenomenon, data already presented must be briefly reviewed. It has been shown, for example, that *Rhizopus tritici* when grown on a nutrient solution makes the substrate considerably more acid. *Rhizopus nigricans*, on the other hand, renders the substrate less acid. In the case of the former species when growing on sweet potato decoction, the solution is not made acid enough to cause maceration of the cells, so that this can not account for any of the maceration noted.

In order to determine if the acid produced under natural conditions in decaying potatoes bore any relation to the rapidity of decay, a series of experiments were performed in which the rate of maceration by the expressed juice was measured. A number of sweet potatoes were inoculated by the "well method" with *Rhizopus tritici* and *R. nigricans*. After a definite length of time the juice was pressed from the decayed portion and the hydrogen-ion concentration determined. The time required to macerate raw sweet-potato disks by the enzym suspended in the solution was also obtained. Control flasks in which the juice was boiled to inactivate the enzym were run in every case. The results of these experiments showed that the sweet-potato disks were decayed in considerably shorter time in the juice from potatoes decayed by *R. tritici* than in the juice from those decayed by *R. nigricans*—that is, in 1.5, 4, 2, and 2.5, 4.75, and 5.5 hours respectively, or in an average of 2½ and 4¼ hours.

The hydrogen-ion concentrations of the expressed juice were as follows: *R. tritici*, 4.008 and 4.082; *R. nigricans*, 5.575 and 5.145. The P_H values of the juice of sound sweet potatoes have been found to vary from 5.0 to 6.0 in a considerable number of tests. The results show that *R. tritici* increases considerably the acidity of the cell sap while *R. nigricans* causes little or no change. The juice from the potatoes decayed by *R. tritici* macerated raw sweet potato disks in almost one-half the time required by the juice from those decayed by *R. nigricans*. If, however, enough acid was added to the juice from potatoes decayed by the latter to make it as acid as that from potatoes decayed by the former, the sweet-potato disks were macerated in the two solutions in exactly the same length of time. Previous investigations have shown that the maximum rate of maceration is obtained when the P_H value of the system is between 3.0 and 4.0; that when it reaches 5 or beyond, the action of the enzym is considerably retarded. In other words, the acid in the system appears to function as a co-enzym and serves to hasten the reaction. Judging from these results, it seems that the feeble macerating action of *R. nigricans* when grown in nutrient solutions may be due, in part at least, to its inability to produce acid; also that it would decay sweet potatoes equally as rapidly as *Rhizopus tritici* if it were not for the fact that it fails to provide optimum conditions by the production of an acid.

LIGHT

The studies on the effect of light on the vital activities of *R. tritici* were more or less preliminary to some of the investigations which will be detailed later. The original purpose of this study was to determine if light was in any way connected with the failure of *R. tritici* to produce pectinase in Czapek's nutrient solution, or with the production of acids in this solution in sufficient quantity to cause maceration identical with that caused by the fungus when grown on sweet potato decoction.

The investigations included the influences of light, on (1) the hydrogen-ion concentration of the substrate. (2) the rate of maceration by the

enzym secreted into the solution, (3) the dry weight of fungous materials produced, (4) fruiting, and (5) the amount of glucose consumed.

The methods employed in these experiments are briefly as follows: Czapek's modified nutrient solution (*15*) was employed, using 20 per cent dextrose as a source of carbon. Thirty cc. of this solution were placed in each of 210 100-cc. Erlenmeyer flasks, 100 of which, after being inoculated, were held in the dark; 100 were inoculated and held in the light; and 10 were left uninoculated as controls. The flasks were all placed on a laboratory table near a north window. A wooden frame was placed over each set of 100 flasks. The flasks in the dark were covered with three thicknesses of a good grade of satine, and held in place on the top by means of plate glass. Air was admitted at the sides, and the light excluded by packing the black cloth closely against the flasks. The top of the frame over the flasks in the light was covered only by the plate glass but the sides were wrapped with white cloth, making them in every way comparable to the sides of the rack covered with black cloth except for the color of the cloth, thus affording the same opportunity for aeration and temperature fluctuations in both sets.

Twenty flasks of each set were removed at the end of 3, 4, 5, 6, and 7 days. The solution from each set was combined into one sample. The sugar present in this sample was determined by means of a Fric saccharimeter. Another portion of the solution was utilized in maceration tests according to methods detailed elsewhere (*4*). The mycelium from each set, after being washed carefully in running water, was collected into one crucible and its dry weight determined. The results from duplicate experiments are shown in Tables II and III.

An examination of Tables II and III reveals no material difference in the results obtained which might be attributed to the influence of light. There was a slight difference in fruiting, but this was not marked. It is interesting to note in this connection that other investigators have found that there is no general agreement among fungi with respect to the influence of light on their growth and fruiting. Lendner (*7*) found considerable disagreement among different members of the Mucorineae and some of the conidial forms of the ascomycetes, while Levin (*8*) showed that total darkness completely suppressed the formation of or greatly reduced the production of pycnidia in some of the Sphaeropsidales. Investigations in still another group of fungi (Agaricaceae) by Maire and de Laroquette (*10*) indicated that here also light has no constant influence on fructification.

The hydrogen-ion concentration of the solutions was considerably increased and the results show that the acid present was sufficient to cause dissolution of the middle lamellae. It was found that after some of the solution on which the fungus had grown was neutralized by the addition of NaOH, it no longer macerated raw disks. There was no difference in the time required to macerate raw sweet-potato disks in the steamed and unsteamed solutions on which the fungus had grown. It will also be seen that there was no maceration in the control solutions, or in the solutions in which the fungus had grown. It seems quite evident from these results that no pectinase was formed, otherwise there would have been some difference in the time required to macerate the disks in the steamed and unsteamed inoculated solutions. What maceration took place in the solutions must be attributed wholly to the acid or to some other substance besides an enzym.

TABLE II.—*Showing the influence of light on the development of Rhizopus tritici*

Time after inoculation.	Light.						Dark.						Uninoculated controls.		
	P_H.	Amount of dextrose.	Rate of maceration by the solution.		Dry weight of mycelium.	Fruiting.	P_H.	Amount of dextrose.	Rate of maceration by the solution.		Dry weight of mycelium.	Fruiting.	P_H.	Amount of dextrose.	Rate of maceration by the solution.
			Not steamed.	Steamed 15 minutes.					Not steamed.	Steamed 15 minutes.					
Days.		*Per cent.*			*Gm.*			*Per cent.*			*Gm.*			*Per cent.*	
3	2.18	15.5	Considerable in 48 hours.	Same as not steamed.	0.0327	None	2.08	15.2	Slight, in 48 hours.	Same as not steamed.	0.0418	None	4.23	15.9	None.
4	1.84	14.75	Well advanced in 48 hours.	do	0.0855	do	1.84	14.65	Nearly complete in 48 hours.	do	0.0842	do	4.39	15.9	Do.
5	1.81	14.7	Complete in 24 hours.	Complete in 24 hours.	0.0939	do	1.81	14.45	Complete in 24 hours.	do	0.0956	do	4.38	15.9	Do.
6	1.82	14.6	do	do	0.0944	do	1.80	14.45	do	do	0.105	do	4.40	16.1	Do.
7	1.82	14.8	do	do	0.094	do	1.82	14.7	do	do	0.1014	do	4.21	16.1	Do.

TABLE III.—*Showing the influence of light on the development of Rhizopus tritici*

Time after inoculaton.	Light.						Dark.						Uninoculated controls.		
	P_H.	Amount of dextrose.	Rate of maceration by the solution.		Dry weight of mycelium.	Fruiting.	P_H.	Amount of dextrose.	Rate of maceration by the solution.		Dry weight of mycelium.	Fruiting.	P_H.	Amount of dextrose.	Rate of maceration by the solution.
			Not steamed.	Steamed 15 minutes.					Not steamed.	Steamed 15 minutes.					
Days.		*Per cent.*			*Gm.*			*Per cent.*			*Gm.*			*Per cent.*	
3	2.12	15.25	None in 48 hours.	None in 48 hours.	0.0413	None	1.90	14.6	Complete in 24 hours.	Complete in 24 hours.	0.0729	None	4.65	15.5	None in 48 hours.
4	1.86	14.8	Complete in 24 hours.	Complete in 24 hours.	0.0889	Some	1.82	14.3	do	do	0.0975	Some	4.59	15.6	Do.
5	1.82	14.8	do	do	0.0871	Considerable	1.82	14.3	do	do	0.098	Considerable	4.78	15.6	Do.
6	1.82	14.9	do	do	0.0899	Slightly more than in dark.	1.82	14.5	do	do	0.0947	Slightly less than in light.			
7	1.82	14.9	do	do	0.0887	Same as in dark.	1.82	14.7			0.0959	Same as in light.	4.64		None in 48 hours.

The change in the amount of dextrose was not large in any case, being approximately the same in the cultures held in the light and in the dark. The weight of the mycelium was not characteristically different, although a little more on an average was actually produced in the dark than in the light. One outstanding feature of these results to which attention should be directed is the short time required for the fungus to reach its maximum growth. If the dry weight of the mycelium produced in 3 days is compared with that produced at the end of succeeding days, it will be seen that the fungus has practically completed its growth in 4 days. The hydrogen-ion concentration of the substrate increases very little after the fourth day. There is also very little decrease in the sugar consumed after that time, which shows that the organism grows very rapidly at first.

The above results show that *R. tritici* greatly increases the hydrogen-ion concentration when grown on Czapek's nutrient solution and that the acidity reaches its maximum in from 3 to 4 days. Additional investigations which will not be given in detail have shown that the solution still remains acid at the end of 10 days and does not tend to become alkaline, as is the case with solutions on which some other organisms have grown. Since *R. nigricans* is a species of great economic importance, similar experiments were carried out with it, the results of which are shown in Table IV.

TABLE IV.—*Showing the P_H value of the solution of sweet-potato decoction on which R. nigricans had grown for different lengths of time*

	Period of growth (hours).								
	0	7	24	31	48	55	76	96	240
R. nigricans:									
Inoculation solution	5.48	5.48	5.07	5.02	5.20	5.22	5.93	6.46	6.15
Control	5.48	5.48	5.48	5.48	5.45	......	5.45	......	5.43
R. tritici:									
Inoculation solution	5.05	5.08	4.44	3.88	3.62	......	3.70	3.59	......
Control	5.05	......	......	......	......	......	5.07	......	......

The solution upon which *Rhizopus nigricans* had grown showed slight increases in acidity at the end of 24 and 31 hours, after which the hydrogen-ion concentration began to decrease, reaching a final concentration in 4 days, considerably less than that of the original solution.

INFLUENCE OF BOTRYTIS CINEREA ON HYDROGEN-ION CONCENTRATION

Botrytis cinerea Pers. has been used in experiments in which it was found to produce a substance capable of dissolving the middle lamellae so that coherence of the cells was completely lost. Smith (*14*), Brown (*2*), de Barry (*1*), Nordhausen (*13*), Kissling (*5*), and others have made a study of the parasitism of this fungus and its mode of action. The results obtained by these different investigators are not entirely in harmony. Smith, for example, claimed that in old mycelium of *Botrytis cinerea* as much as 2 per cent oxalic acid was produced, which he suspected to be responsible for some of the conditions noted. He appears to have discovered a twofold action on lettuce tissue, one which results

in the killing of the cells and another which brings about a softening of the tissues. The first is caused, he claims, by the acid, and the latter by one or more enzyms. Brown, on the other hand, was unable to demonstrate a killing action independent of that caused by the macerating principle and apparently leans to the view that the entire action, killing and macerating, is due to one and the same substance. Smith made no mention of the age of the mycelium which he used. Brown used mycelium only 1 or 2 days old, so that it is not likely that acids in any considerable amount were present in it. On the other hand, it is probable that an acid was produced in Smith's cultures if he used mycelium several days or weeks old. The writers have shown that *Rhizopus tritici* does not produce an appreciable amount of acid until after about 3 days. They are aware, however, that this is no indication of what *Botrytis cinerea* may do.

The writers included a study of *Botrytis cinerea* in their investigations for three principal reasons. First, it has been studied by a number of investigators and shown to produce a substance capable of dissolving this middle lamellae of the cells of a number of different hosts; second, it has been shown by the writers that species of Rhizopus, which are not parasitic on the sweet potato, produce a weak macerating enzym when grown in culture. In other words, it was found that certain organisms which were not normally parasites on the sweet potato would produce an enzym or some substance when grown in culture which would act upon the middle lamellae. Whether or not this was a characteristic of all fungi was not known. Therefore, it was decided to make such a study of another organism which had already been shown to produce a cell-wall dissolving enzym and which was not a true parasite of the sweet potato. Third, it seemed necessary to determine whether an acid was produced which would cause a maceration of the cells, as the writers showed to be the case when *Rhizopus tritici* was grown on Czapek's solution.

Several different media were employed because the results of previous investigations showed that *Rhizopus tritici*, although it formed a pectinase when grown on sweet potato decoction, would not do so when cultivated on Czapek's nutrient solution with glucose as a source of carbon. In order to partially eliminate the influence which the substrata might exert on the production of pectinase by *Botrytis cinerea*, six decoctions of vegetable origin, namely, string bean, prune, Irish potato, carrot, turnip, and sweet potato, three synthetic media, Czapek's, Richard's, and Pfeffer's solutions, and beef bouillon were employed. The organisms were grown in 100 cc. Erlenmeyer flasks on 30 cc. of media. There were a number of flasks of each media. After inoculation the cultures were incubated in the dark at 28° C. for 7 days. At the end of the growth period the contents of all the flasks of one set were collected into one compound sample. The hydrogen-ion concentrations of the uninoculated controls and the solutions on which the fungus had grown were then determined. The mycelium was saved from one set of experiments and its macerating power determined. On some of the solutions the growth was very poor and no hyphae were obtained.

The results as expressed in Table V show that *Botrytis cinerea* increased the hydrogen-ion concentration in some solutions and decreased it in others. String bean and Irish potato decoctions were changed to a point on the alkaline side of neutrality, the action of the enzym being

perhaps thereby retarded. The results of these hydrogen-ion determinations show that the final alkalinity or acidity of a solution depends not alone upon the fungus growing upon it, but in part, at least, upon its composition.

TABLE V.—*Showing the change in hydrogen-ion concentration in the solutions, and the rate of maceration by the enzym in the mycelium and in the solution on which it grew*

Media.	Experiment 1.		Experiment 2.		Experiment 3.			
	P_H.		P_H.		P_H.		Maceration.	
	Control.	Inoculated.	Control.	Inoculated.	Control.	Inoculated.	Solution.	Hyphae.
String bean decoction.	4.83	8.21	4.82	7.62	4.82	8.26	None in 48 hours....	Complete in 32 hours.
Prune decoction......	(1)	(1)	3.85	3.68	3.85	4.62	Complete in 24 hours	....
Irish potato decoction.	5.52	8.21	5.52	8.08	5.52	8.17	Slight in 48 hours...	Complete in 6 hours.
Carrot decoction......	3.24	5.73	4.88	5.42	4.88	5.55	Complete in 48 hours	Complete in 32 hours.
Turnip decoction.....	4.81	5.98	4.76	6.23	4.76	7.99	Some in 48 hours....	Complete in 24 hours.
Sweet-potato decoction.	(1)	(1)	4.89	4.61	4.89	4.62	Complete in 48 hours	Complete in 32 hours.
Czapek's solution....	4.03	2.65	4.05	2.69	4.05	2.52	Complete in 24 hours	Complete in 24 hours.
Pfeffer's solution.....	3.53	2.92	3.41	3.14	3.41	3.05	Complete in 48 hours	....
Richard's solution...	(1)	(1)	3.27	3.12	3.27	3.17	None in 48 hours....	....
Beef bouillon.........	(1)	(1)	8.15	8.13	8.15	8.22	.....do...............	....

[1] No growth.

There was no maceration of raw sweet-potato disks in portions of the solutions which had been steamed before testing them, except in Czapek's solution, which had a final P_H of 2.52. There was a slight loss of coherence of the cells in this solution in 48 hours, which was probably due to the acid present in the solution. A careful study of the table reveals the fact that in no case, neither in the solution nor in the hyphae, was a vigorous cell-wall dissolving enzym produced. Only in one case, that of the hyphae grown in Irish potato decoction, was maceration completed in less than 24 hours, which shows for the most part that a pectinase capable of acting upon sweet-potato tissue is only feebly produced by *Botrytis cinerea.*

These data can not be used to substantiate or refute the work of Smith, who claims to have demonstrated the production of oxalic acid. Undoubtedly, the age of the culture would exercise some influence upon the amount of acid produced and Smith does not state the length of time he allowed his cultures to grow. The writers were unable to detect the presence of acid in the mycelium of a 7 days' growth. The distilled water in which a quantity of mycelium was soaked for 3 hours at room temperature had a P_H value of 6.8, which is practically neutral.

Apparently *Botrytis cinerea* is very similar in certain physiological characteristics to some of the species of Rhizopus, which, although not normally parasitic on the sweet potato, produce an enzym, perhaps in a small amount, capable of dissolving the middle lamellae of sweet-potato disks. *Botrytis cinerea* does not secrete a substance which will act on sweet-potato tissue to the same degree that it will on some other vegetable tissues, as shown by Brown and others. Is there then a variety of pectinases secreted by different fungi which is more or less specific for the tissue of certain hosts? Since it is impossible to isolate these enzyms,

an unqualified answer to this question can not be given. All eviden along this line must be based on the action which the enzyms in solutio have on cellular structures. It is a well-known fact that in the decay of certain vegetables and fruits by such organisms as Rhizopus and Botrytis the cells lose their coherence, probably as the result of the action of an enzym. It is also well known that some fungi will produce such an action on some hosts and not on others, which suggests the possibility that the macerating principle produced by one fungus may be quite different from that produced by another.

SUMMARY

(1) The influence exercised by the growth of 11 different species of Rhizopus on the acidity of the substrate (Czapek's nutrient solution) was studied. It was found that two species (*Rhizopus nigricans* and *R. microsporus*) make the solution less acid. All the other species make it more acid.

(2) The expressed juice from sweet potatoes decayed by *R. tritici* was found to be more acid than that from potatoes decayed by *R. nigricans.* Raw sweet-potato disks suspended in the juice of potatoes decayed by *R. tritici* were macerated in a shorter time than those immersed in juice from potatoes decayed with *R. nigricans.* If the juice in both cases is brought to the same hydrogen-ion concentration, the disks are macerated in the same length of time.

(3) Light exercised very little influence on the production of pectinase.

(4) Changes in hydrogen-ion concentration of the substrate induced by *Botrytis cinerea* were studied for several different media. The results show that the hydrogen-ion concentration of some substrates is increased, while that of others is decreased.

(5) *Botrytis cinerea,* although not normally a sweet potato storage-rot organism, produces a small amount of pectinase capable of dissolving the middle lamellae of raw sweet-potato disks when grown in artificial cultures.

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GROWTH-PROMOTING VALUE OF THE PROTEINS OF THE PALM KERNEL, AND THE VITAMIN CONTENT OF PALM-KERNEL MEAL[1]

By A. J. FINKS, *Assistant Chemist*, and D. BREESE JONES, *Chemist in Charge, Protein Investigation Laboratory, Bureau of Chemistry, United States Department of Agriculture*

INTRODUCTION

The oil palm, *Elaeis guineensis*, grows naturally along the West Coast of Africa, but it is also being introduced into other localities. The fruit consists of a kernel or nut inclosed by a hard shell of varying thickness. This hard shell is surrounded by an outer fleshy, oily pericarp from which the commercial palm oil is produced. Palm kernels contain from 45 to 50 per cent of oil, which is removed by expression or by solvent extraction. The resulting oil cake or meal is used as cattle feed. Until comparatively recently, the separation of the oily pericarp from the kernels was made by the natives by means of crude methods, whereby the kernels were subjected to conditions which would tend to denature the proteins, thus rendering them unsuitable for isolation and chemical study.

During the war large quantities of palm kernels were shipped into this country, and it seemed, for a time at least, that they would offer a cheap and nutritious article in such quantity as to become a significant factor in our feedstuff industry. Although there is little or no importation of palm kernels into this country at present, the enormous supply of this source of important feedstuff makes it very desirable to secure as much knowledge as possible regarding its nutritive value.

Palm-kernel meal as a feed for cattle has been given a high rating. Crowther[2] has reported that in digestion experiments with sheep it was found that palm-kernel cake ranks among the most digestible of the stock feeds, and is more valuable than cottonseed meal. Hooper and Nutter,[3] in feeding experiments with milch cows, found that palm-kernel meal could be used with advantage as a supplement to corn for the production of milk.

So far as we are aware, no previous work has been done to ascertain the nutritive value of the proteins of the palm kernel when fed to animals as the sole source of protein in a diet adequate with respect to the other essential dietary factors.

The palm-kernel meal used for the feeding experiments described in this paper was a commercial product which had been prepared by the solvent process. It contained 19.44 per cent of protein ($N \times 6.25$).

[1] Accepted for publication May 2, 1923.
[2] CROWTHER, Charles. PALM KERNEL CAKE. *In* Jour. Bd. Agr. [London], v. 23, p. 734-749. 1916.
[3] HOOPER, J. J., and NUTTER, J. W. FEEDING TRIALS OF VELVET-BEAN FEED, PALM-KERNEL MEAL AND VARIOUS GRAIN MIXTURES, FOR DAIRY COWS. Ky. Agr. Exp. Sta. Circ. 23, p. 31-38, illus. 1918.

The results obtained in these studies show that palm-kernel meal, when fed at a protein intake level of 15.5 per cent of the diet, furnishes protein adequate for the normal growth of albino rats, and that when the meal constitutes as much as 40 per cent of the diet it does not furnish sufficient vitamin A to prevent xerophthalmia, or of vitamin B to provide for normal growth. However, since the meal used for these experiments was a commercial product from which the oil had been removed by the solvent process, and since the history of the kernels prior to the extraction of the oil, involving the treatment they were subjected to in removing the oily pericarp, is unknown, the vitamin deficiencies of the commercial meal used in these experiments may not be characteristic of the fresh, untreated palm kernels.

GROWTH-PROMOTING VALUE OF PALM-KERNEL PROTEINS

Albino rats weighing from 40 to 70 gm. were fed on a diet containing 80 parts of palm-kernel meal, equivalent to 15.5 per cent of protein. The diet was satisfactory with respect to the constituents other than protein. Vitamin B was supplied by a daily allowance of 80 mgm. of a yeast vitamin preparation made according to the method described by Osborne and Wakeman,[4] and 0.3 gm. of cod liver oil furnished vitamin A. In all the experiments the yeast preparation and cod liver oil were given separately from the rest of the diet. The composition of the diet [5] and curves representing the rates of growth are given in chart 1.

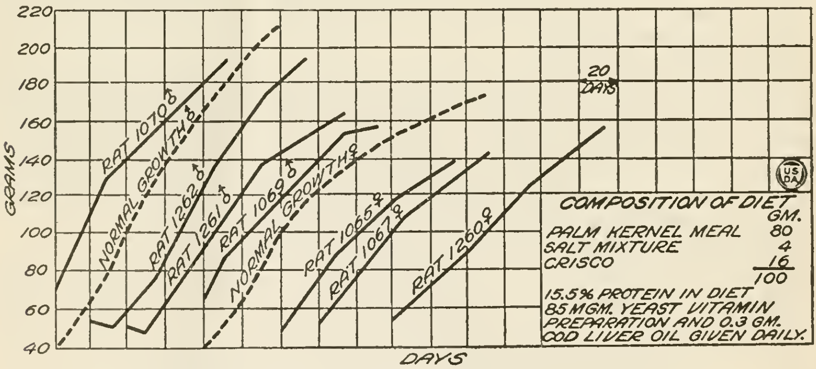

CHART 1.—Curves showing growth-promoting value of palm-kernel proteins.

These curves show that the proteins of the palm kernel are adequate for normal growth when they constitute the sole source of protein at a 15.5 per cent level intake in a diet otherwise nutritionally satisfactory.

EXPERIMENTS SHOWING VITAMIN B DEFICIENCY IN PALM-KERNEL MEAL

Chart 2 shows the results obtained when the rats received no vitamin B other than that which may have been supplied by the palm-kernel meal when this constituted 25 per cent of the diet. In order to insure a sufficient quantity of adequate protein, 15 parts of purified casein [6] were

[4] OSBORNE, Thomas B., and WAKEMAN, Alfred J. EXTRACTION AND CONCENTRATION OF THE WATER-SOLUBLE VITAMINE FROM BREWERS' YEAST. *In* Jour. Biol. Chem., v. 40, p. 383–394, 4 charts. 1919.

[5] For the composition of the salt mixture see: OSBORNE, T. B., and MENDEL, L. B. THE USE OF SOY BEAN AS FOOD. *In* Jour. Biol. Chem., v. 32, p. 374. 1917.

[6] The casein and "crisco" used in the experiments for the study of the vitamin content of the meal had been treated and found to be devoid of both vitamin A and vitamin B. The casein was purified by extraction with 1 per cent acetic acid, followed by several extractions with 50 per cent alcohol, and finally by extraction with ether. The crisco was treated by passing air through it at 100° C. for 6 to 7 hours.

incorporated in the diet. These animals grew at a fair rate during the first 20 or 25 days, then began to decline. At points marked X, the animals were given daily 85 mgm. of yeast vitamin preparation to which

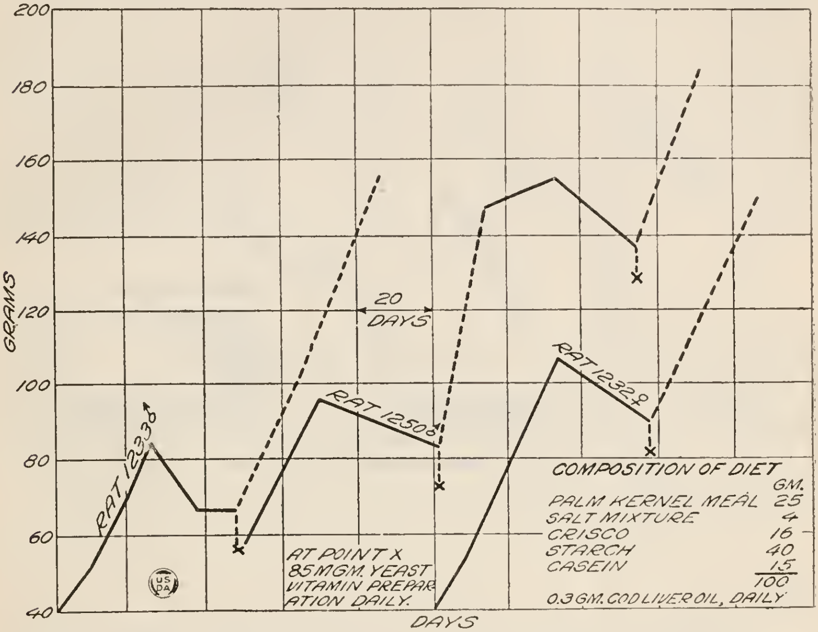

CHART 2.—Curves showing vitamin B deficiency with 25 per cent palm-kernel meal in diet.

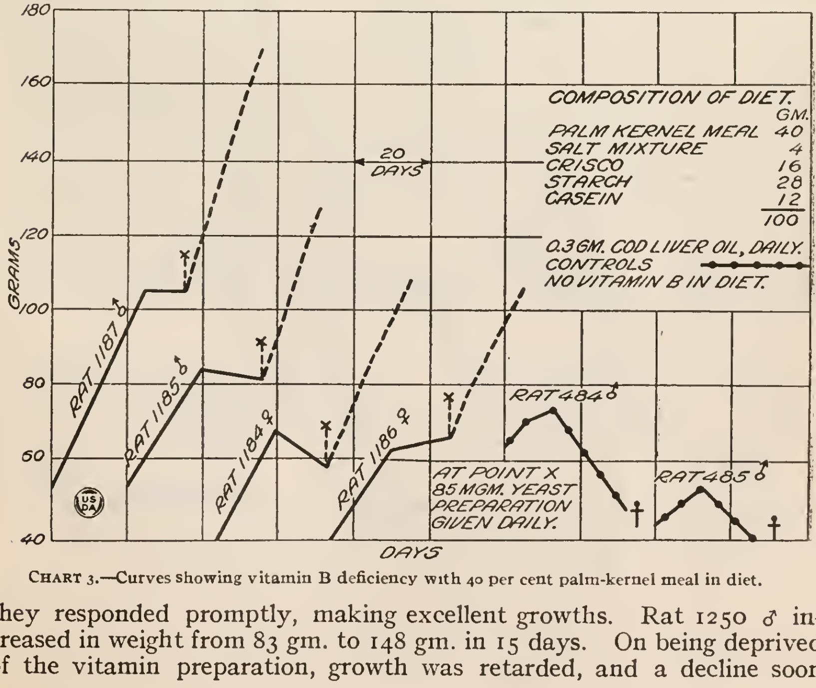

CHART 3.—Curves showing vitamin B deficiency with 40 per cent palm-kernel meal in diet.

they responded promptly, making excellent growths. Rat 1250 ♂ increased in weight from 83 gm. to 148 gm. in 15 days. On being deprived of the vitamin preparation, growth was retarded, and a decline soon

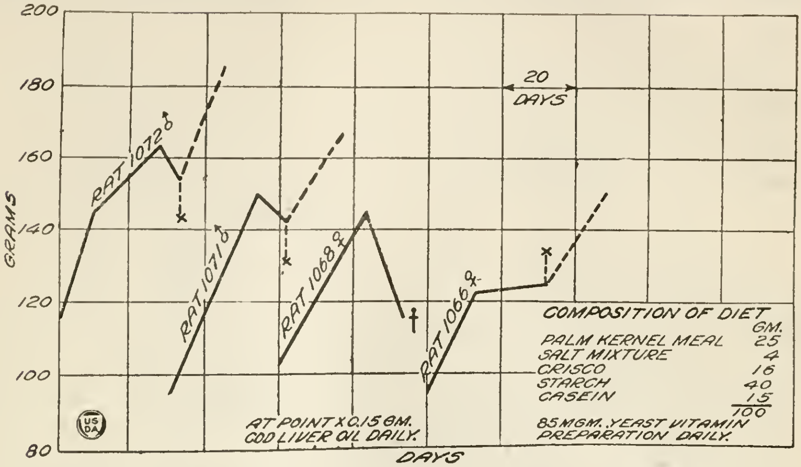

CHART 4.—Curve showing vitamin A deficiency with 25 per cent palm-kernel meal in diet.

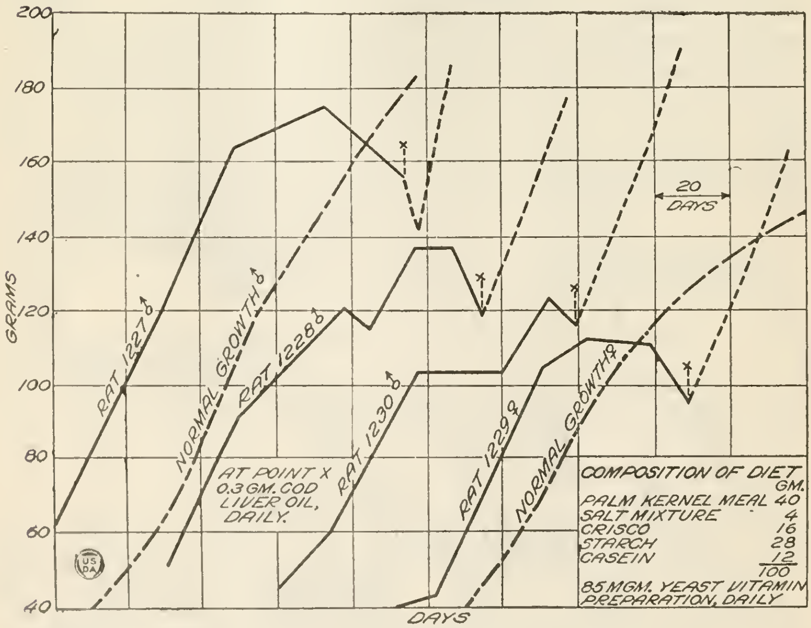

CHART 5.—Curve showing vitamin A deficiency with 40 per cent palm-kernel meal in diet.

followed. Resupplying the animal with the vitamin preparation was again followed by a prompt recurrence of growth.

The curves in chart 3 show that when the quantity of palm-kernel meal was increased to 40 per cent of the diet, there was still a deficiency of vitamin B. That the meal contained some of this vitamin, however, becomes apparent on comparing these curves with those of control rats 484 and 485, which received purified casein as the sole source of protein in their diet. These rats received a diet containing the same percentage of protein as did the others whose growth curves are given in this chart. The control rats made a decidedly slower initial growth and in two weeks began to decline rapidly. It is also to be noted that the animals whose diet contained 40 per cent of the meal did not show decline as soon as those on the 25 per cent meal diet.

EXPERIMENTS SHOWING VITAMIN A DEFICIENCY

The curves in chart 4 show that 25 parts of palm-kernel meal in the diet does not furnish sufficient vitamin A. After an initial growth of 24 to 25 days the rats began to decline and developed xerophthalmia. The addition of 0.3 gm. of cod liver oil promptly cured the eye trouble and enabled the animals to grow at a fair rate. Rat 1068 had declined to such an extent that it failed to respond to cod liver oil.

Where 40 per cent of the meal was used in the diet (chart 5), the decline in growth and onset of xerophthalmia occurred considerably later than in the case of the rats which received the 25 per cent meal ration.

SUMMARY

The proteins of palm-kernel meal were found to be adequate for the normal growth of young rats, when fed in a diet balanced with respect to the other dietary factors. The meal constituted 80 per cent of the diet, which is equivalent to 15.5 per cent of protein.

Forty per cent of palm-kernel meal did not furnish sufficient vitamin A to prevent xerophthalmia, and a like quantity did not provide sufficient vitamin B for normal growth. Since the meal used for these experiments was a commercial product obtained as a residue from the nuts after removal of the oil by the solvent process, the results obtained with reference to vitamin content, however, may not necessarily apply to the fresh, untreated palm-kernel nut.

Inasmuch as it is this treated commercial product which is available for the feeding of live stock, the vitamin values given in this article are of more practical value than would be those found for the fresh kernels.

EFFICIENCIES OF PHOSPHATIC FERTILIZERS AS AFFECTED BY LIMING AND BY THE LENGTH OF TIME THE PHOSPHATES REMAINED IN PORTO RICAN SOILS [1]

By P. L. Gile, *formerly Chemist*, and J. O. Carrero, *Assistant Chemist, Porto Rico Agricultural Experiment Station*

INTRODUCTION

Notwithstanding the fact that hundreds of field experiments have been conducted to determine the relative values of the different phosphatic fertilizers, only very few accurate quantitative data on this subject have been obtained. Failure to obtain data has, in most instances, been due to the usual difficulties attending tests of this kind, but in a number of cases it has been due to failure to ascertain whether any of the phosphates were applied in excess of the crop's requirements. Experiments conducted during the last 15 years with the different phosphates in pot cultures have been more fruitful of results in that they have demonstrated quite clearly several general factors influencing the efficiencies of the various phosphates.

The experiments of Prianischnikov (*21*),[2] Kossowitsch (*13*), Jordan (*9*), Chirikov (*2*, *3*), Truog (*31*), Wrangell (*32*), and Bauer (*1*) show that different crops vary greatly in their abilities to utilize the rock phosphates. The last four investigators named suggest that these differences in "feeding power" are correlated with the quantities of lime that are assimilated by the crop, or with the relative quantities of lime and phosphoric acid that are absorbed.

That the character of the soil affects the efficiences of the phosphates is evident from many field trials and from the work of Kossowitsch (*13*) and Gedroiz (*4*).

Carbonate of lime markedly decreases the efficiencies of bone meal and rock phosphate, but, according to the results of Kellner and Böttcher (*10*, *11*), Söderbaum (*27*, *28*, *29*), Nagaoka (*19*), Prianischnikov (*21*, *22*, *23*, *24*), Kossowitsch (*12*), Liechti (*14*), and Mitscherlich (*16*, *17*), it does not affect the efficiencies of basic slag, dicalcium phosphate, or the water-soluble phosphates.

Investigations by Prianischnikov (*21*, *24*), Kossowitsch (*12*), Söderbaum (*29*, *30*), and Mitscherlich and Simmermacher (18) show that the insoluble phosphates are appreciably more efficient when ammonium sulphate is applied with the phosphate than when sodium nitrate is used as the source of nitrogen.

The effect which carbonate of lime exerts on the efficiencies of the phosphates has become a matter of much practical importance since liming has become a general practice. It is especially important to know the effect of applications of lime such as would be required to satisfy the lime requirement of different soils. The work cited above, however, does not

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[1] Accepted for publication May 2, 1923.
[2] Reference is made by number (italics) to "Literature cited," p. 193–194.

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give much information on this point, since many of the experiments were performed in quartz sand and in other experiments the quantities of lime applied bore no particular relation to the lime requirements of the soils. Also, very little work has been done with a view to ascertaining the rate at which the different phosphates gain or lose in efficiency by remaining in different soils. This is a question of considerable moment in the fertilization of long-time crops, such as fruit trees, sugar cane, and pineapples.

The experiments reported in this paper were conducted to determine the relative efficiencies of acid phosphate, double superphosphate, basic slag, bone meal, and finely ground rock phosphate when used under different conditions in eight types of Porto Rican soils. The results are of general interest, however, in showing quantitatively how the various phosphates are affected by remaining in different soils and by applications of lime which are sufficient to satisfy the "lime requirements" of the soils.

EXPERIMENTAL METHODS

The relative efficiencies of the phosphates were calculated, not from the magnitudes of the increases produced by equal quantities of phosphoric acid, but from the relative quantities of phosphoric acid required to produce the same increase in yield. For example, if 2 gm. of phosphoric acid from floats produced the same increase as 0.5 gm. of phosphoric acid from acid phosphate, the floats phosphoric acid was considered as being 25 per cent as efficient as the acid phosphate phosphoric acid. In order to follow this method of comparison, it was necessary to have in each experiment a series of pots receiving increasing quantities of phosphoric acid from the standard material, acid phosphate. A curve was plotted from the yields of these pots to show the extent to which growth would be increased by any quantity of acid phosphate.[3]

As pointed out in another publication, (*6*) it is believed that this method of comparison is more likely to give accurate results than is the usual method because it is not only not based on any assumption concerning the law of minimum, but it is accurate, irrespective of how growth increases with increasing quantities of the elements in minimum; and it does not necessitate an analysis of the crop.

Basic slag, bone meal, and floats or finely ground rock phosphates, were compared on the basis of their total phosphoric acid content, while acid phosphate and double superphosphate were compared on the basis of their "available" or ammonium-citrate-soluble, phosphoric acid. The efficiencies of the various phosphates are expressed as compared with the efficiency shown by acid phosphate when the latter was applied in the unlimed soil immediately before planting was done.[4]

In the larger experiments the differently treated pots were replicated five times or more. The probable error of the average result for each treatment was therefore fairly low—less in most cases than 4 per cent of the yield. The regularity of the curves showing the increases in growth produced by increasing applications of acid phosphate also confirmed the accuracy of the results.

Millet (*Setaria italica*), which readily responds to phosphoric acid, was used as the test crop. As soon as the seeds had matured and the

[3] A small preliminary test was conducted with each kind of soil to determine approximately the maximum quantity of phosphoric acid to which the crop would respond.

[4] In Experiment VII the yields produced by acid phosphate in the limed soil were taken as the standard for comparison, since in this soil acid phosphate was very ineffective without lime.

heads had turned yellow the plants were cut. This stage of maturity was reached in 36 to 48 days after the plumules had broken the ground, according to the season in which the plants were grown. Both green and oven-dried weights of the heads and stalks from each pot were obtained, but for the sake of conciseness only the oven-dried weights of the combined heads and straw are reported. The ratio of heads to straw appeared to be constant for plants of a given weight in any one experiment, irrespective of the source of phosphoric acid used. Where the larger quantities of available phosphates were used, the growth of the plants was fully equal to that made under good field conditions.

Glazed earthenware pots were used in some of the tests, and tin pots that had been coated with tar paint were used in others. Each container had a capacity of 5 gallons, and both kinds of containers gave equally good results. During the day the pots were kept on trucks in a wire inclosure (5 meshes to the inch) and at night and during heavy rains they were run into a glasshouse. The order of the trucks was shifted daily and the order of the pots on each truck was changed every few days to insure uniform conditions of growth.

As soon as an experiment was started the moisture content of each soil was made up to about 60 per cent of its maximum water-holding capacity, and was kept constant by weighing. When the plants attained considerable size the weights of the pots plus the soil were taken daily, and as the plants became larger, allowance was made for the added weights of the plants. Transpired water was replaced by rainwater containing only 17 parts per million of total solids. The plan of the experiment and the methods of comparison were such, however, that appreciable impurities should not have affected the accuracy of the result (*6*).

The phosphates were thoroughly mixed with the first 4 inches of soil in the pots after the various applications had been made up to the same weight by the addition of silica sand. Whenever the phosphatic fertilizers were added to some of the pots in an experiment, the soil in all the other pots was stirred in a similar manner so that it might be in a uniform mechanical condition in all the pots at the time of planting. The sodium nitrate, ammonium sulphate, and potassium salts were added in solution, half of the total quantity being incorporated with the soil before planting was done, and the remainder when the plants were somewhat less than half-grown. Half the nitrogen was derived from sodium nitrate and half from ammonium sulphate, in order that the efficiencies of the insoluble phosphates might not be appreciably affected by the unassimilated acid and alkaline residues of the nitrogen salts.

In the limed series sufficient lime (air-slaked lime containing some carbonate) was added to each pot to satisfy the lime requirement of the soil as determined by the Veitch method, the lime being thoroughly incorporated with the volume of soil in each pot three or four days before the phosphates were added.

Analyses of the five different phosphates used in the experiments are given in Table I.

TABLE I.—*Analyses of the phosphatic materials which were used in the vegetation tests*

Material.	Total phosphoric acid (P_2O_5).	Water-soluble phosphoric acid.	Citrate-soluble phosphoric acid.
	Per cent.	*Per cent.*	*Per cent.*
Acid phosphate	21.33	14.98	17.27
Rock phosphate	30.63	....	.73
Bone meal	26.03	....	....
Basic slag	17.90	....	[1] 9.57
Double superphosphate	45.70	37.73	44.27

[1] Fourteen per cent available in 2 per cent citric acid.

Soil samples were taken from parts of the field that had not been manured or fertilized for many years, at least. From 5 to 6 tons of each type of soil were used in the experiments. Table II shows the chemical composition of the soils in which the phosphates were tested.

TABLE II.—*Chemical composition of soils in which the phosphates were tested*[a]

Soil constituents.	Soil No. 1524.	Soil No. 1257.	Soil No. 1529.	Soil No. 1578.	Soil No. 1716.	Soil No. 213.	Soil No. 1796.	Soil No. 1810.	Soil No. 1811.
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Silica (SiO_2)	86.17	73.92	97.37	51.32	92.99	53.34	54.41	....	....
Titanium oxid (TiO_2)	.55	.60	.33	1.16	.39	.85	1.13	....	....
Ferric oxid (Fe_2O_3)	2.11	4.48	.62	11.71	1.80	12.45	11.05	....	....
Alumina (Al_2O_3)	5.49	12.36	.78	22.47	2.22	13.03	20.45	....	....
Manganese dioxid (MnO_2)	.056	.35	.018	.37	.174	.16	.06	....	....
Lime (CaO)	.28	.27	.26	.30	.19	2.77	.45	....	....
Magnesia (MgO)	.37	.25	.09	.15	.12	8.27	.62	....	....
Potash (K_2O)	.33	.54	.13	.36	.15	1.54	.54	....	....
Soda (Na_2O)	.06	.07	Trace.	.47	.02	1.44	.29	....	....
Phosphoric acid (P_2O_5)	.02	.09	.01	.43	.03	.15	.06	....	....
Sulphur trioxid (SO_3)	.12	.10	.05	....	.12	.07	.16	....	....
Loss on ignition	5.28	7.53	1.32	12.66	1.88	6.32	12.00	....	....
Total	100.84	100.56	100.98	101.40	100.08	100.39	101.22	....	....
Nitrogen (N)	.15	.14	.06	.26	.05	.03	.21	....	....
Organic matter	3.93	3.80	2.64	3.64	1.75	....	....	....	....
Lime requirement[b]	.103	.108	.056	.176	.006	....	.196	00.272	0.075

[a] The samples were analyzed by the Bureau of Soils, U. S. Department of Agriculture, by the method of the Association of Official Agricultural Chemists.
[b] By Veitch method; CaO required expressed as percentage of weight of soil.

All the soils except No. 213 are important agriculturally, and when planted with Citrus or pineapples are heavily fertilized. No. 1524 is a brown, fine sandy loam from Bayamon; No. 1257 is a reddish brown, fine sandy loam from Pueblo Viejo; No. 1529 is a gray sand from Toa Baja; No. 1578 is a clay loam from the Vega Baja-Manati section, and derived from a limestone; No. 1716 is a grayish brown, fine sandy loam from Barceloneta; No. 213 is a medium sand, a riverwash; No. 1796 is red clay from Mayaguez (5); and No. 1810 and 1811 are red and black clay, respectively, from Rio Piedras.

EFFICIENCIES OF THE PHOSPHATES AS AFFECTED BY THE SOIL

Experiments I to VIII, inclusive, were similar in plan, but in each instance a different type of soil was used. The soil in half the series of pots in each experiment received sufficient lime to satisfy its requirement by the Veitch method, and that in the remaining pots was left unlimed. The various phosphates were applied to some of the pots in both the limed and unlimed series six weeks before planting was done, and to the remainder the day before planting. The essential details of each of the eight experiments are given in Table III.

TABLE III.—*Conditions of Experiments I to VIII, inclusive*

Experiment No.	Soil No.	Quantity of moisture-free soil per pot.	Optimum water content of soil.[1]	Sodium nitrate added per pot.	Ammonium sulphate added per pot.	Potassium sulphate added per pot.	Number of plants grown per pot.	Number of days plants grew.
		Pounds.	*Per cent.*	*Gm.*	*Gm.*	*Gm.*		
I	1810	31.2	40.0	5.6	4.0	5.3	15	46
II	1811	37.9	30.0	5.6	4.0	5.3	15	48
III	1716	42.5	12.3	5.6	4.0	5.3	14	40
IV	1529	41.6	13.0	5.6	4.0	5.3	18	43
V	1257	42.6	22.0	6.3	4.5	6.0	15	38
VI	1578	37.8	30.0	5.3	3.8	5.0	18	43
VII	1796	31.1	33.0	5.6	4.0	5.3	14	40
VIII	1524	41.6	29.0	8.4	6.0	8.0	21	36

[1] Expressed in percentage of dry soil.

Table IV shows the relative efficiencies of the various phosphates in both the limed and unlimed soils when the phosphates were applied immediately before and six weeks before planting.

TABLE IV.—*Efficiencies of the five different phosphates in the first eight experiments*

PHOSPHATES MIXED WITH SOIL IMMEDIATELY BEFORE PLANTING

	Kind of phosphate applied.	Phosphoric acid (P_2O_5) applied per pot.	Soil not limed. Oven-dry yield of individual pots. (Heads, leaves, and stalks.)						Soil not limed. Average oven-dry yield.	Soil not limed. Relative efficiency of the phosphates. (Acid phosphate applied immediately before planting in unlimed soil=100.)	Soil limed. Oven-dry yield of individual pots. (Heads, leaves, and stalks.)						Soil limed. Average oven-dry yield.	Soil limed. Relative efficiency of the phosphates. (Acid phosphate applied immediately before planting in unlimed soil=100.)
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
Experiment I. Soil No. 1524.	No phosphate	....	4.0	3.8	3.3	3.7	4.6	....	....	....	2.4	2.5	2.0	2.6	2.4	....	....	....
	Do	....	3.2	3.7	....	....	....	....	3.8	....	2.3	2.4	....	....	....	....	2.4	....
	Acid phosphate	0.30	7.8	8.7	9.9	10.2	9.7	....	9.3	100	9.0	9.2	9.6	9.8	10.9	....	9.7	122
	Do	.60	17.9	16.8	25.7	17.1	16.2	....	18.7	100	17.2	15.4	11.8	16.1	16.3	....	15.4	90
	Do	1.00	23.0	23.4	25.1	22.2	24.8	....	23.7	100	22.8	22.6	23.1	22.8	23.6	....	23.0	106
	Do	1.75	33.4	34.2	30.5	33.1	....	....	32.8	100	31.8	32.2	35.5	32.9	32.3	....	32.9	126
	Do	2.75	34.5	37.9	37.1	35.2	36.7	....	36.3	100	37.6	36.4	36.5	41.4	37.4	....	37.9	....
	Floats	4.00	12.4	12.7	14.5	11.6	11.8	....	12.6	10	3.3	2.7	3.9	3.6	4.2	....	3.5	2
	Bone meal	1.25	28.5	27.4	29.6	23.0	29.2	....	27.5	105	17.7	14.8	17.7	14.0	19.4	....	16.7	46
	Basic slag	1.00	27.2	24.1	24.7	26.3	23.2	....	25.1	111	23.7	22.4	21.1	19.4	20.9	....	21.5	93
	Double superphosphates	1.00	22.2	18.7	23.3	20.4	24.2	....	21.8	85	20.5	19.1	22.6	19.1	22.2	....	20.7	87
	PHOSPHATES MIXED WITH SOIL 6 WEEKS BEFORE PLANTING																	
	Acid phosphate	0.60	13.8	11.6	14.0	14.7	11.1	....	13.0	70	9.0	9.0	11.2	12.0	10.1	....	10.3	63
	Do	1.75	30.7	28.1	26.9	30.0	31.0	....	29.3	84	25.7	22.5	26.2	29.9	28.3	....	26.5	77
	Floats	4.00	14.3	13.2	15.6	15.5	13.4	....	14.4	12	3.3	3.3	4.1	3.4	4.5	....	3.7	2
	Bone meal	1.25	24.9	21.2	24.6	27.2	21.6	....	23.9	81	14.1	11.7	13.8	14.3	14.0	....	13.6	39
	Basic slag	1.00	17.8	16.8	19.9	19.0	21.4	....	19.0	62	13.4	15.1	14.7	16.2	15.9	....	15.1	53
	Double superphosphates	1.00	20.1	23.3	21.2	17.4	19.0	....	20.2	72	14.9	16.2	17.1	19.5	17.3	....	17.0	59

PHOSPHATES MIXED WITH SOIL IMMEDIATELY BEFORE PLANTING

Experiment II. Soil No. 1578.	No phosphate	...	0.2	0.2	0.2	0.1	0.3	...	...	...	0.1	0.2	0.2	0.2	0.2	...	...	...
	Do	...	.2	.2	...	...	...	...	0.2	...	.2	...	...	...	...	...	0.2	...
	Acid phosphate	0.4	6.6	4.6	5.6	5.3	4.9	...	5.4	100	5.5	4.0	2.7	2.2	2.2	...	3.3	60
	Do	.8	12.5	14.0	13.3	10.1	12.0	...	12.4	100	7.9	7.5	9.5	6.9	9.5	...	8.3	70
	Do	1.2	23.1	23.6	22.5	20.4	18.4	...	21.6	100	19.3	20.0	16.2	12.7	12.9	...	16.2	80
	Do	1.8	30.3	28.3	33.3	28.1	30.3	...	30.0	100	19.9	27.9	25.7	27.3	21.1	...	24.4	77
	Do	3.6	38.5	43.5	46.5	41.8	36.2	...	41.3	100	27.5	39.2	31.3	40.2	34.8	...	34.6	71
	Floats	6.0	19.5	21.7	20.8	23.7	21.5	...	21.4	20	1.1	.8	1.2	.8	1.0	...	1.0	1
	Bone meal	1.5	17.0	18.4	21.1	20.5	18.1	...	19.0	72	1.4	5.0	3.5	5.3	...	...	3.8	19
	Basic slag	1.2	20.4	15.3	22.9	19.0	22.6	...	20.0	94	8.6	8.7	11.2	11.0	14.9	...	10.9	60
	Double superphosphate	1.2	25.5	23.6	25.3	20.5	14.4	...	21.9	101	16.1	16.3	11.2	16.9	20.5	...	16.2	80

PHOSPHATES MIXED WITH SOIL 6 WEEKS BEFORE PLANTING

Experiment II. Soil No. 1578.	Acid phosphate	0.8	4.4	5.3	4.7	9.1	6.7	...	6.0	54	3.4	3.3	2.8	4.0	6.7	...	4.0	38
	Do	1.8	15.0	18.8	22.5	20.5	19.5	...	19.3	61	11.6	13.7	13.4	14.2	18.4	...	14.3	49
	Floats	6.0	19.4	18.9	18.1	16.1	20.1	...	18.5	18	.8	.5	1.0	1.4	.7	...	.9	1
	Bone meal	1.5	13.2	17.2	16.6	13.8	14.9	...	6.0	60	1.9	2.8	2.5	3.7	2.1	...	2.6	12
	Basic slag	1.2	8.7	10.1	9.6	11.3	11.1	...	10.2	57	5.7	7.1	7.1	6.4	6.4	...	6.5	38
	Double superphosphate	1.2	8.8	10.3	8.7	13.1	9.9	...	10.2	57	9.2	7.5	9.2	9.3	7.5	...	8.5	48

PHOSPHATES MIXED WITH SOIL IMMEDIATELY BEFORE PLANTING

Experiment III. Soil No. 1257.	No phosphates	...	24.5	23.6	24.4	32.0	13.7	27.7	...	...	25.9	25.3	28.6	24.2	22.9	28.9	...	...
	Do	...	28.6	...	...	...	...	...	24.9	...	29.3	...	...	...	...	...	26.4	...
	Acid phosphate	0.2	29.0	24.0	31.9	28.8	26.0	27.5	27.9	100	...	...	...	...	...	...	...	...
	Do	.4	29.8	26.3	33.8	31.0	32.1	35.7	31.5	100	...	...	...	...	...	...	...	...
	Do	.8	35.0	30.9	33.6	34.6	38.3	35.9	34.7	100	34.5	34.0	30.7	31.4	38.6	38.2	34.6	75
	Do	1.2	37.6	29.5	37.1	38.3	39.8	42.0	37.4	100	...	...	...	...	...	...	...	...
	Do	2.4	48.9	42.2	46.3	48.3	45.6	51.0	47.0	100	...	...	...	...	...	...	...	...
	Floats	4.0	34.6	30.7	34.4	35.1	41.5	34.4	35.1	22	26.4	27.9	31.3	23.8	27.2	31.9	28.1	3
	Bone meal	1.0	32.4	28.2	30.6	33.3	34.3	35.3	32.4	51	29.0	31.5	31.8	29.5	34.8	31.9	31.4	31
	Basic slag	.9	31.9	33.3	33.8	34.2	36.9	30.6	33.5	71	29.7	29.5	30.7	29.9	37.3	36.2	32.2	40
	Double superphosphate	.7	35.3	30.0	34.4	31.4	35.3	32.7	33.2	87	32.3	32.7	32.9	27.9	38.3	29.8	32.3	51

TABLE IV.—*Efficiencies of the five different phosphates in the first eight experiments*—Continued

PHOSPHATES MIXED WITH SOIL 2 WEEKS BEFORE PLANTING

	Kind of phosphate applied.	Phosphoric acid (P_2O_5) applied per pot.	Soil not limed. Oven-dry yield of individual pots. (Heads, leaves, and stalks.)						Soil not limed. Average oven-dry yield.	Soil not limed. Relative efficiency of the phosphates. (Acid phosphate applied immediately before planting in unlimed soil=100.)	Soil limed. Oven-dry yield of individual pots. (Heads, leaves, and stalks.)						Soil limed. Average oven-dry yield.	Soil limed. Relative efficiency of the phosphates. (Acid phosphate applied immediately before planting in unlimed soil=100.)
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
Experiment III. Soil No. 1257.	Acid phosphate	0.4	26.7	22.2	27.6	34.9	32.7	24.4	28.1	52	....	....	....	....	....	....	....	....
	Do	.8	27.2	28.2	29.9	33.5	34.7	30.6	30.7	45	....	....	....	....	....	....	....	....
	Floats	4.0	29.7	28.9	38.6	35.6	35.6	32.7	33.5	16	....	....	....	....	....	....	....	....
	Bone meal	1.0	27.3	28.6	35.8	35.2	29.7	35.4	32.0	46	....	....	....	....	....	....	....	....
	Basic slag	.9	27.8	23.1	34.4	29.1	41.2	30.7	31.1	42	....	....	....	....	....	....	....	....
	Double superphosphate	.7	25.5	19.1	34.4	30.6	34.6	32.6	29.5	41	....	....	....	....	....	....	....	....
	PHOSPHATES MIXED WITH SOIL 6 WEEKS BEFORE PLANTING																	
	Acid phosphate	0.4	20.8	24.8	29.8	29.9	33.9	28.2	27.9	50	....	....	....	....	....	....	....	....
	Do	.8	28.1	30.3	29.0	28.4	34.2	35.3	30.9	46	33.1	34.9	33.0	31.9	35.5	32.6	33.5	56
	Floats	4.0	30.3	37.5	32.9	38.9	38.2	36.8	35.8	24	24.5	29.0	34.1	28.5	28.1	30.4	29.1	5
	Bone meal	1.0	24.0	29.9	29.4	34.4	31.0	37.3	31.0	38	25.4	34.1	33.9	31.1	33.6	31.2	31.6	32
	Basic slag	.9	28.0	29.7	32.5	35.5	32.5	34.5	32.1	52	26.1	32.6	39.8	35.4	35.3	34.3	33.9	55
	Double superphosphate	.7	28.2	28.6	30.7	31.1	29.5	40.0	31.4	57	29.3	34.9	36.0	32.1	31.8	32.3	32.7	56

Experiment No. IV. Soil No. 1810.

PHOSPHATES MIXED WITH SOIL IMMEDIATELY BEFORE PLANTING																	
No phosphates	....	11.3	8.5	11.6	9.3	11.2	....	....	....	6.4	7.0	8.3	7.6	8.1	....	....	....
Do	....	11.4	12.2	....	....	....	....	10.8	....	7.7	5.7	....	....	....	....	7.3	....
Acid phosphate	0.4	19.4	20.1	14.9	19.1	16.0	....	17.9	100	14.2	13.8	15.1	12.9	15.8	....	14.4	100
Do	.8	21.7	22.3	22.4	24.3	22.9	....	22.7	100	17.0	16.7	21.7	16.7	23.9	....	19.2	100
Do	1.5	27.2	25.8	27.4	25.1	28.5	....	26.8	100	25.4	28.6	27.6	25.0	27.6	....	26.8	157
Do	2.5	30.1	25.9	33.4	36.2	31.2	....	31.4	100	35.4	34.5	39.5	36.5	36.1	....	36.4	....
Do	3.5	35.7	35.3	35.8	29.5	29.4	....	33.1	....	38.6	37.7	39.7	44.1	41.1	....	40.2	....
Floats	4.0	25.6	24.1	25.8	28.1	26.5	....	26.0	34	7.8	8.8	7.6	8.8	7.6	....	8.1	5
Bone meal	1.8	21.8	24.1	22.5	21.5	21.8	....	22.3	43	19.2	16.4	16.0	17.2	16.8	....	17.1	35
Basic slag	1.5	28.3	24.0	27.1	26.8	24.7	....	26.2	92	23.0	24.3	24.3	26.4	24.5	....	24.5	117
Double superphosphate	1.5	25.1	24.7	28.4	23.1	25.8	....	25.4	83	28.1	33.3	26.2	27.7	27.7	....	28.6	....
PHOSPHATES MIXED WITH SOIL 6 WEEKS BEFORE PLANTING																	
Acid phosphate	0.8	16.3	20.0	11.3	16.8	19.3	....	16.7	41	14.3	14.5	14.7	13.4	13.9	....	14.2	49
Do	2.5	26.8	26.7	25.4	24.8	30.5	....	26.8	80	26.6	....	28.5	27.9	27.2	....	27.6	98
Floats	4.0	23.4	27.6	27.1	23.4	27.2	....	25.7	32	8.4	8.8	10.8	12.1	12.5	....	10.5	5
Bone meal	1.8	20.3	27.0	22.1	20.7	23.4	....	22.7	44	14.2	15.4	11.8	13.9	12.7	....	13.6	20
Basic slag	1.5	19.2	19.9	20.4	20.7	24.1	....	20.9	37	17.8	16.4	18.1	17.1	14.9	....	16.9	41
Double superphosphate	1.5	21.8	22.3	20.0	20.7	20.8	....	21.1	44	17.6	20.8	16.9	19.3	18.9	....	18.7	51

Experiment No. V. Soil No. 1811.

PHOSPHATES MIXED WITH SOIL IMMEDIATELY BEFORE PLANTING																	
No phosphates	....	6.5	5.1	5.9	5.8	6.0	....	....	....	4.2	5.1	5.4	5.7	5.3	....	....	....
Do	....	5.9	7.1	....	....	....	....	6.0	....	5.4	5.4	....	....	....	....	5.2	....
Acid phosphate	0.4	16.3	16.1	17.6	19.4	19.8	....	17.8	100	17.6	16.3	13.0	18.2	17.2	....	16.5	95
Do	0.8	23.0	24.5	23.5	25.6	22.7	....	23.9	100	24.0	24.9	23.8	23.6	20.9	....	23.4	103
Do	1.5	41.2	35.4	36.7	24.2	36.4	....	36.8	100	41.5	38.5	33.0	31.0	38.0	....	36.4	95
Do	2.5	45.5	49.7	45.7	47.7	46.6	....	47.0	100	39.5	43.5	52.8	42.9	46.8	....	45.1	94
Do	3.5	51.7	56.1	53.5	51.7	56.2	....	53.8	100	49.1	54.1	53.1	53.3	50.5	....	52.0	95
Floats	6.0	26.4	23.9	24.7	23.7	27.7	....	25.3	15	13.8	13.9	16.4	12.4	14.3	....	14.2	5
Bone meal	3.0	20.0	24.5	28.1	36.6	29.4	....	27.7	33	22.5	26.5	24.6	30.9	25.0	....	25.9	32
Basic slag	1.5	30.1	28.8	28.7	28.5	29.6	....	29.1	69	22.2	24.4	27.2	23.3	26.0	....	24.6	59
Double superphosphate	1.5	39.9	39.9	38.1	34.7	36.2	....	37.8	97	29.1	32.1	34.8	29.3	34.7	....	32.0	81
PHOSPHATES MIXED WITH SOIL 6 WEEKS BEFORE PLANTING																	
Acid phosphate	0.8	16.8	19.8	19.5	16.9	19.4	....	18.5	55	16.8	17.1	18.5	15.7	13.6	....	16.2	48
Do	2.5	37.1	29.1	47.7	38.8	38.9	....	38.3	59	39.2	39.0	37.5	35.4	35.8	....	37.4	59
Floats	6.0	27.8	20.0	23.9	22.5	24.4	....	23.7	13	12.8	11.3	12.4	10.8	12.2	....	11.9	4
Bone meal	3.0	20.0	31.6	24.7	20.7	33.9	....	26.2	30	20.6	21.8	21.3	17.4	20.6	....	20.3	21
Basic slag	1.5	23.6	24.0	27.5	23.9	23.3	....	24.5	55	20.5	20.2	20.9	21.2	20.5	....	20.7	43
Double superphosphate	1.5	24.0	22.7	29.8	28.7	33.2	....	27.7	66	25.4	26.8	25.1	27.0	29.2	....	26.7	65

TABLE IV.—*Efficiencies of the five different phosphates in the first eight experiments*—Continued

PHOSPHATES MIXED WITH SOIL IMMEDIATELY BEFORE PLANTING

Experiment No. VI. Soil No. 1716.

Kind of phosphate applied.	Phosphoric acid (P_2O_5) applied per pot.	Soil not limed. Oven-dry yield of individual pots. (Heads, leaves, and stalks.)						Soil not limed. Average oven-dry yield.	Soil not limed. Relative efficiency of the phosphates. (Acid phosphate applied immediately before planting in unlimed soil=100.)	Soil limed. Oven-dry yield of individual pots. (Heads, leaves, and stalks.)						Soil limed. Average oven-dry yield.	Soil limed. Relative efficiency of the phosphates. (Acid phosphate applied immediately before planting in unlimed soil=100.)
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
No phosphate	....	20.9	20.3	24.1	19.9	21.7	....	....	....	21.7	20.4	20.7	22.6	21.6	....	....	....
Do	....	24.0	23.7	....	....	....	....	22.1	....	18.0	21.9	....	....	....	....	21.0	....
Acid phosphate	0.15	28.1	29.0	29.1	27.2	31.2	....	28.9	100	28.6	22.9	25.7	28.3	29.7	....	27.0	90
Do	.30	25.5	31.0	31.0	31.1	31.9	....	30.1	100	29.2	28.4	28.8	33.1	31.5	....	30.2	157
Do	.60	32.5	31.3	37.5	34.3	34.2	....	33.9	100	35.2	29.5	38.3	36.0	35.1	....	34.8	138
Do	.90	36.0	32.2	40.3	36.6	37.4	....	36.5	100	37.4	35.6	39.5	34.4	36.5	....	36.7	....
Do	1.50	39.8	35.8	37.6	37.8	35.5	....	37.3	....	38.2	35.7	34.9	36.1	31.8	....	35.3	....
Floats	3.00	25.6	30.7	27.5	29.5	34.9	....	29.6	8	23.4	25.7	29.4	27.2	28.8	....	26.9	4
Bone meal	.90	33.5	34.4	36.3	33.8	33.8	....	34.4	72	31.9	30.6	33.7	33.0	36.2	....	33.1	70
Basic slag	.60	33.3	37.0	31.4	33.8	35.3	....	34.2	105	35.3	30.9	34.5	34.6	34.1	....	33.9	120
Double phosphate	.60	33.1	34.1	33.7	32.9	36.0	....	34.0	100	35.4	31.9	29.0	37.0	37.8	....	34.2	128
PHOSPHATES MIXED WITH SOIL 6 WEEKS BEFORE PLANTING																	
Acid phosphate	0.30	30.1	28.2	28.9	33.4	29.5	....	30.0	96	30.5	26.4	30.4	29.3	30.9	....	29.5	113
Do	.90	35.2	34.5	29.8	32.4	34.7	....	33.3	61	35.8	34.9	32.0	37.8	40.3	....	36.2	....
Floats	3.00	30.8	29.9	29.2	28.3	28.2	....	29.3	7	30.1	30.7	29.8	30.1	29.9	....	30.1	13
Bone meal	.90	32.9	33.3	34.5	32.1	33.2	....	33.2	60	34.3	33.3	34.7	34.1	34.2	....	34.1	83
Basic slag	.60	37.2	30.8	33.5	36.3	36.2	....	34.8	118	35.2	36.1	37.5	35.2	32.2	....	35.2	148
Double superphosphate	.60	33.2	35.5	33.3	34.3	30.8	....	33.4	93	34.4	35.5	34.1	31.5	34.7	....	34.0	123

PHOSPHATES MIXED WITH SOIL IMMEDIATELY BEFORE PLANTING																	
Experiment No. VII. Soil No. 1529.																	
No phosphate	......	4.2	3.4	3.7	3.1	5.2	......	......	......	3.2	3.1	3.9	2.0	4.9	......	......	......
Do	......	4.9	5.7	......	......	......	......	4.3	......	3.6	3.7	......	......	......	......	3.5	......
Acid phosphate	0.30	18.0	15.5	15.1	11.3	21.1	......	16.2	[1] 78	17.6	17.7	18.1	21.4	18.7	......	18.7	[1] 100
Do	.60	19.5	19.1	23.9	23.1	24.8	......	22.1	[1] 68	21.5	23.5	29.8	29.8	25.4	......	26.0	[1] 100
Do	1.00	27.4	22.3	27.7	25.3	28.2	......	26.2	[1] 58	30.3	32.9	29.7	28.2	32.2	......	30.7	[1] 100
Do	1.75	28.6	23.4	24.5	24.7	28.3	......	25.9	[1] 32	35.4	31.0	32.6	33.3	34.3	......	33.3	[1] 100
Do	2.75	31.0	25.1	29.9	23.6	28.3	......	27.6	[1] 25	28.3	33.4	33.5	31.5	30.9	......	31.5	......
Floats	3.50	27.8	28.9	28.3	28.3	29.6	......	28.6	[1] 79	4.3	5.6	5.3	7.4	6.1	......	5.7	[1] 1
Bone meal	1.25	32.3	29.5	31.7	30.6	32.5	......	31.3	[1] 78	22.6	21.1	28.3	26.3	21.3	......	23.9	[1] 34
Basic slag	1.00	32.4	29.8	34.4	31.6	29.7	......	31.6	[1] 100	28.7	31.0	28.1	32.5	32.0	......	30.5	[1] 98
Double superphosphate	1.00	27.2	26.0	24.9	25.9	25.0	......	25.8	[1] 56	33.7	30.6	32.3	34.4	29.3	......	32.1	[1] 140
PHOSPHATES MIXED WITH SOIL 6 WEEKS BEFORE PLANTING																	
Acid phosphate	0.60	16.0	17.8	21.0	18.0	16.6	......	17.9	[1] 45	15.8	17.0	18.0	19.0	22.3	......	18.4	[1] 49
Do	1.75	28.4	25.1	26.8	26.4	25.1	......	26.4	[1] 33	31.6	31.7	29.4	32.4	32.6	......	31.5	[1] 69
Floats	3.50	24.4	22.1	26.5	22.6	31.4	......	25.4	[1] 16	3.9	4.7	4.1	7.5	7.4	......	5.5	[1] 1
Bone meal	1.25	29.3	29.7	32.4	28.2	27.4	......	29.4	[1] 66	20.3	19.3	19.5	25.5	16.4	......	20.2	[1] 29
Basic slag	1.00	28.5	24.3	31.0	30.9	32.9	......	29.5	[1] 83	27.5	26.6	23.6	31.1	22.7	......	26.3	[1] 63
Double superphosphate	1.00	22.3	17.1	22.2	23.4	18.7	......	20.7	[1] 35	19.1	23.7	21.1	28.6	29.3	......	24.4	[1] 54
PHOSPHATES MIXED WITH SOIL IMMEDIATELY BEFORE PLANTING																	
Experiment No. VIII. Soil No. 1796.																	
No phosphate	......	2.2	2.3	3.7	2.8	3.1	......	......	......	2.6	4.4	3.5	3.4	2.9	......	......	......
Do	......	2.7	2.8	......	......	......	......	2.8	......	2.7	3.4	......	......	......	......	3.3	......
Acid phosphate	0.2	19.1	20.9	22.1	22.5	24.4	......	21.8	100	11.6	12.7	15.3	17.4	16.8	......	14.8	60
Do	.4	33.7	35.7	36.9	37.6	39.0	......	36.6	100	24.2	24.0	23.9	29.6	25.9	......	25.5	61
Do	.8	47.5	48.4	50.7	52.3	46.0	......	49.0	100	32.3	37.3	39.1	34.6	37.3	......	36.1	49
Do	1.2	51.3	57.0	55.5	59.4	56.6	......	56.0	100	51.8	43.9	53.9	52.3	52.1	......	50.8	73
Do	2.0	67.7	68.2	59.6	62.7	59.3	......	63.5	100	56.8	52.4	61.1	58.4	57.1	......	57.2	64
Floats	2.5	51.7	53.6	55.7	60.3	54.6	......	55.2	46	8.1	7.7	12.2	9.4	10.8	......	9.6	3
Bone meal	1.2	50.3	57.4	61.0	51.6	57.0	......	55.5	98	30.6	18.2	30.5	32.3	29.5	......	28.2	23
Basic	.8	37.9	43.5	43.2	50.6	45.4	......	44.1	80	37.5	30.1	37.1	34.3	37.9	......	35.4	47
Double superphosphate	.8	39.8	49.8	47.4	45.1	45.9	......	45.6	86	27.1	33.7	39.1	36.6	38.9	......	35.1	47
PHOSPHATES MIXED WITH SOIL 6 WEEKS BEFORE PLANTING																	
Acid phosphate	0.4	19.9	18.2	19.7	19.9	20.1	......	19.6	45	12.8	16.2	14.8	18.5	12.8	......	15.0	30
Do	1.2	39.0	41.3	41.5	55.3	42.8	......	44.0	53	31.4	38.2	41.9	36.5	42.4	......	38.1	36
Floats	2.5	35.7	51.1	39.9	46.8	44.3	......	43.6	25	7.0	12.2	10.4	6.0	7.7	......	8.7	2
Bone meal	1.2	37.1	45.0	47.4	49.0	45.4	......	44.8	55	20.2	21.8	27.3	19.0	20.2	......	21.7	16
Basic slag	.8	25.7	32.8	31.4	33.4	36.7	......	32.0	43	25.4	27.4	27.8	29.9	30.1	......	28.1	35
Double superphosphate	.8	26.5	33.1	35.5	37.5	38.4	......	34.2	46	28.3	25.4	30.0	28.9	28.6	......	28.2	35

[1] In the case of soil No. 1529, the efficiency of the phosphates is compared with that of acid phosphate in the *limed* soil applied immediately before planting taken as 100, since acid phosphate had a low efficiency in this soil without lime.

The plants responded very markedly to phosphoric acid in all the soils, the yields being increased from 2 to 20 times by the largest quantities of acid phosphate applied. In all soils, both limed and unlimed, the larger applications of acid phosphate produced relatively less increments in yield than did the smaller applications, which, of course, is the normal effect of increasing any limiting factor. In the case of soil No. 1810, however, the larger applications of acid phosphate were markedly less efficient in the unlimed soil than they were in the limed soil, although the smaller applications were about equally efficient in both series. In soil No. 1529 all the quantities of acid phosphate applied were less efficient in the unlimed series than in the limed, the differences in efficiencies in the two series being greater with the larger quantities of acid phosphate applied. Obviously, the maximum yields in these two soils were not to be obtained with acid phosphate as a source of phosphoric acid without the use of lime.

Table V summarizes the results obtained in these experiments.

TABLE V.—*Summary of the results obtained in Experiments I to VIII, inclusive, phosphates mixed with limed and unlimed soil*

UNLIMED SOIL

Time of application.	Kind of phosphate applied.[1]	Soil No. 1524.	Soil No. 1578.	Soil No. 1257.	Soil No. 1810.	Soil No. 1811.	Soil No. 1716.	Soil No. 1529.	Soil No. 1796.	Soil No. 213.[2]
Immediately before planting.	Acid phosphate	100	100	100	100	100	100	52	100	100
Do	Floats	10	20	22	34	15	8	79	46	4
Do	Bone meal	105	72	51	43	33	72	78	98	27
Do	Basic slag	111	94	71	92	69	105	110	80	59
Do	Double superphosphate	85	101	87	83	97	100	56	86	......
6 weeks before planting.	Acid phosphate	77	58	48	61	57	79	39	49	54
Do	Floats	12	18	24	32	13	7	16	25	2
Do	Bone meal	81	60	38	44	30	60	66	55	21
Do	Basic slag	62	57	52	37	55	118	83	43	......
Do	Double superphosphate	72	57	57	44	66	93	35	46	......

LIMED SOIL

Time of application.	Kind of phosphate applied.[1]	Soil No. 1524.	Soil No. 1578.	Soil No. 1257.	Soil No. 1810.	Soil No. 1811.	Soil No. 1716.	Soil No. 1529.	Soil No. 1796.	Soil No. 213.[2]
Immediately before planting.	Acid phosphate	111	72	75	119	96	128	100	61	90
Do	Floats	2	1	3	2	5	4	1	3	0
Do	Bone meal	46	19	31	35	32	70	34	23	4
Do	Basic slag	93	60	40	117	59	120	98	47	31
Do	Double superphosphate	87	80	51	......	81	128	140	47	......
6 weeks before planting.	Acid phosphate	70	44	56	74	54	113	59	33	......
Do	Floats	2	1	5	5	4	13	1	2	......
Do	Bone meal	39	12	32	20	21	83	29	16	......
Do	Basic slag	53	38	55	41	43	148	63	35	......
Do	Double superphosphate	59	48	56	51	65	123	54	35	......

[1] The values given for acid phosphate are averages of the results yielded by several different quantities of this material.

[2] The results obtained in the river sand, No. 213, are the averages of several experiments described in a previous publication (*7, p. 25-29*).

Floats (finely ground rock phosphate) was a fairly efficient source of phosphoric acid in some of the soils when no lime was used. It was practically without effect, however, when applied to limed soils immediately before planting was done. The uniformity of the very low efficiencies of floats under these conditions, from 0 to 5 in the different soils, is doubtless significant. In quartz sand, floats usually has about

3 per cent of the efficiency of acid phosphate for the gramineae. It is possible that this value represents the relative efficiencies of floats and acid phosphate in soils which do not contain compounds unsaturated with calcium.

Bone meal varied more in efficiency than did any other phosphate according to the character of the soil. In soils No. 1796, 1524, and 1529 it had an efficiency equal to, or greater than, acid phosphate when no lime was applied, while in soils No. 1810, 1811, and 213 it was only about one-third as effective as acid phosphate. In all the limed soils except No. 1716 it was rather an ineffective source of phosphoric acid.

When the results obtained with basic slag are considered, it should be borne in mind that this material was compared with the other phosphates on the basis of its total phosphoric acid content, although it is sold on the basis of its "available" phosphoric acid. In the slag used in the experiments 78 per cent of the total phosphoric acid was soluble in 2 per cent citric acid, that is, "available" by the Wagner method. Considered on the basis of its available phosphoric acid, basic slag was more efficient, on the whole, than was acid phosphate, since in the nine unlimed soils it averaged 87 per cent as efficient as acid phosphate when applied immediately before planting was done. Basic slag was also very effective when it was applied six weeks before planting and when it was used in conjunction with lime.

The efficiency of the phosphoric acid in double superphosphate was practically the same as that in acid phosphate.

It might be expected that the efficiencies of certain phosphates would depend to a considerable extent upon those qualities of the soil which are indicated by the lime requirement. Apparently, however, this is not so. Table VI shows the lime requirements of the soils and the efficiencies of the different phosphates in the soils when applied immediately before planting without the use of lime. The efficiency of each phosphate is expressed relative to 100 for the efficiency of acid phosphate applied under the same conditions, except in the case of soil No. 1529. In the case of this soil the efficiency of each phosphate is expressed relative to 52 for the efficiency of acid phosphate, since acid phosphate was relatively ineffective in this soil without lime. (See Table V.)

TABLE VI.—*Showing the relation between the lime requirements of the soils and the relative efficiencies of the phosphates*

Soil No.	Lime (CaO) required to neutralize soil.[1]	Efficiency of—			
		Floats.	Bone meal.	Basic slag.	Double superphosphates.
1810	0.272	34	43	92	83
1796	.196	46	98	80	86
1578	.176	20	72	94	101
1257	.108	22	51	71	87
1524	.103	10	105	111	85
1811	.075	15	33	69	97
1529	.056	79	78	100	56
1716	.006	8	72	105	100

[1] Expressed in percentage of soil.

It is apparent from Table VI that none of the phosphates varied in efficiency directly with the lime requirement of the soil. On the whole, floats was far more efficient in soils with high lime requirements than in those with low requirements, but there is no exact correspondence between this measure of the soil's acidity and the efficiency of floats.

Lack of correspondence may be due in part to the fact that the efficiencies of all the phosphates are expressed relative to the efficiency of acid phosphate. The table would in such a case merely show the effect of the lime requirement properties of the soil in altering relative efficiencies. The effect on absolute efficiencies—that is, on the quantity of phosphoric acid required to produce a given increase in crop—can not be judged, since the experiments with the various soils were carried on at different times. Moreover, the absolute efficiencies of the phosphates would doubtless depend upon the degree of phosphorus deficiency in the soil as well as upon other factors.

EFFICIENCIES OF THE PHOSPHATES AS AFFECTED BY LIMING

The degree to which liming affected the efficiencies of the different phosphates can best be seen if the data given in Table V are presented in another form. Table VII shows the effect of liming on the efficiencies of phosphates, the efficiency of each phosphate in the unlimed soil being taken as 100 and in the limed soil as relative to 100.

TABLE VII.—*Effect of liming on the efficiency of phosphates* [1]

Soil No.	Phosphates applied immediately before planting.					Phosphates applied 6 weeks before planting.				
	Acid phosphate.	Floats.	Bone meal.	Basic slag.	Double superphosphate.	Acid phosphate.	Floats.	Bone meal.	Basic slag.	Double superphosphate.
1524	111	20	44	84	102	91	17	48	85	82
1578	72	5	26	64	79	76	6	20	67	84
1257	75	14	61	56	59	117	21	84	106	98
1810	119	6	81	127	....	121	16	45	111	116
1811	96	33	97	86	84	95	31	70	78	98
1716	128	50	97	114	128	143	186	138	126	132
1529	192	1	44	98	250	151	6	44	76	154
1796	61	6	23	59	55	67	8	29	81	76
213	90	0	15	53	....	....	....	....	....	....
Average [2]	107	17	59	86	108	108	36	60	91	105

[1] The efficiency of each phosphate in the unlimed soil, applied immediately before, or 6 weeks before planting taken as 100; the efficiency of the phosphates in the limed soil expressed comparatively.
[2] Soil No. 213 not included.

Evidently the effect of lime on the efficiency of the phosphate depends largely upon the character of the soil. In three soils liming had little influence on the efficiency of acid phosphate which was applied immediately before planting was done; in three soils, it depressed the efficiency; and in three soils it increased the efficiency of acid phosphate. The action of lime was equally variable in the different soils in the case of basic slag and double superphosphate. Although the efficiencies of floats and bone meal were depressed by lime in all soils except No. 1716, the extent of the depression varied greatly according to the character of the soil.

The average values [5] given in Table VII indicate the varying degree to which the different phosphates were affected by liming. Acid phosphate, basic slag, and double superphosphate were relatively little affected by liming, while bone meal lost about 40 per cent of its efficiency and floats lost approximately 75 per cent. This is in accord with the results obtained by Prianischinikov (*22*) in sand cultures. While the average values probably indicate the average of the results that might be secured on a large number of different soils, they obviously do not permit of the prediction of the effect of lime on phosphates in any particular soil.

It was desirable to know whether the time elapsing between the application of the lime and the application of the phosphate influenced the degree to which lime depressed the efficiency, and likewise whether the quantity of lime applied had any appreciable influence when sufficient lime was already present to satisfy the lime requirement of the soil. An experiment was therefore conducted to gain such information, a soil in which lime had markedly affected the efficiency of bone meal being used for the purpose.

Experiment IX was conducted in the same manner as those previously described. Each pot contained 20.5 kgm. of soil No. 1524 maintained at a water content of 29 per cent of the dry weight. The basic fertilization consisted of 4.2 gm. of sodium nitrate, 3 gm. of ammonium sulphate, and 4 gm. of potassium sulphate per pot. Twenty plants per pot were grown for a period of 43 days. Two different quantitites of lime were tried. One series of pots received the lime six weeks before either planting was done, or phosphates were applied. Another series received the two quantities of lime the day before the phosphates were applied. Acid phosphate and bone meal were both applied immediately before planting was done. Table VIII gives the results of the experiment.

TABLE VIII.—*Effect of quantity of lime and the time it was applied on the efficiency of bone meal in Experiment IX*

Time lime was applied.	Lime (CaO) applied per pot.	Phosphate applied immediately before planting.	Phosphoric acid per pot.	Oven-dry yield of individual pots.			Average oven-dry yield.	Relative efficiency of bone meal and acid phosphate. (Acid phosphate applied 6 weeks before planting to soil with 21.06 gm. CaO=100.)
	Gm.		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
Six weeks before planting	21.06	No phosphate		2.5	3.4	3.6	3.2	
Do	21.06	Acid phosphate	.30	11.9	11.4	11.5	11.6	100
Do	21.06	do	.60	22.1	17.7	15.3	18.4	100
Do	21.06	do	1.00	27.7	29.4	27.6	28.2	100
Do	21.06	do	2.00	41.5	40.6	46.8	43.0	100
Do	21.06	Bone meal	1.25	16.3	14.5	13.2	14.7	34
Do	42.12	No phosphate		3.0	3.1	3.3	3.1	
Do	42.12	Bone meal	1.25	5.5	4.1	4.6	4.7	5
Immediately before planting	21.06	No phosphate		4.4	4.9	5.5	4.9	
Do	21.06	Bone meal	1.25	11.3	15.2	14.2	13.6	26
Do	42.12	No phosphate		2.8	4.3	3.9	3.7	
Do	42.12	Bone meal	1.25	4.9	5.8	4.4	5.0	4

[5] The few results secured with soil No. 213 were not considered when the average values were calculated in order that the latter might be comparable for all the phosphates.

Very little difference was observed in the efficiency of bone meal regardless of whether the lime was applied six weeks or one day before the phosphates were used. The quantity of lime applied, however, had a most pronounced effect. The efficiency of bone meal was almost negligible in the presence of a considerable excess of lime, such as was afforded by the 42.12 gm. per pot.

The data given in Table VII show that applications of lime equivalent to the "lime requirement" of the soil may increase, decrease, or be without appreciable effect on, the efficiencies of acid phosphate, basic slag, and double superphosphate, according to the character of the soil. Such quantities of lime may be expected to reduce the efficiencies of floats and bone meal much more generally and to a greater degree.

Table VIII shows that the quantity of lime applied markedly influences the efficiencies of the phosphates. It would therefore be expected that even on soils such as No. 1529 and 1716 where a certain quantity of lime noticeably increased the efficiencies of acid phosphate, basic slag, and double superphosphate, a larger application of lime would have decreased the efficiencies of these materials.

This conclusion is borne out by the results that are being obtained at the Rhode Island Experiment Station (*8*). A certain quantity of lime on the Kingston soil markedly augments the efficiencies of the phosphates for some crops. Liming beyond a certain point has the reverse effect, however. At least, liming beyond a certain point brings about such a condition in the soil that smaller increases are produced by a given quantity of phosphoric acid from some phosphates.

Although pot and field experiments show that liming affects the efficiency of a phosphate in the sense that a given quantity of the phosphate produces a larger or smaller crop increase on limed soil than on unlimed, results obtained with only one or two kinds of phosphates do not necessarily show that liming affects the quantity of phosphoric acid which is in an assimilable condition. It may be that in certain cases liming is without influence on the assimilability of the phosphoric acid applied and merely corrects or exaggerates a soil condition which is so affecting the growth of the plant that it responds less markedly to an increase in the quantity of assimilable phosphoric acid. The depressing effect of lime observed in the experiments reported in this paper, however, are believed to have been due to the effect of the lime on the quantity of phosphoric acid available to the plant; otherwise, it would hardly be understandable why floats should have had such a low and constant efficiency in the limed soils (Table V) and why the efficiencies of some phosphates were depressed less than others.

EFFICIENCIES OF THE PHOSPHATES AS AFFECTED BY REMAINING IN THE SOIL

The extent to which the efficiencies of the various phosphates were affected by remaining six weeks in the unplanted soils is shown in Table IX. In this table the efficiency of each phosphate applied immediately before planting, to either the limed or the unlimed soil, is expressed as 100, and the efficiency of the material applied six weeks before planting is expressed as relative to 100.

TABLE IX.—*Efficiencies of the phosphates as affected by remaining 6 weeks in the soil* [1]

Soil No.—	Unlimed soil.					Unlimed soil.				
	Acid phosphate.	Floats.	Bone meal.	Basic slag.	Double superphosphate.	Acid phosphate.	Floats.	Bone meal.	Basic slag.	Double superphosphate.
1524	77	120	77	56	85	63	100	85	57	68
1578	58	90	83	61	56	61	100	63	63	60
1257	48	109	75	73	66	74	167	103	138	110
1810	61	94	102	40	53	62	250	57	35	....
1811	57	87	91	80	67	56	80	66	73	80
1716	79	88	83	112	93	88	325	119	123	96
1529	75	20	85	83	63	59	100	85	64	39
1796	49	54	56	54	53	54	67	70	74	74
213	54	50	77	....	....	....	....	....	....	....
Average [2]	63	83	82	70	67	65	140	81	78	75

[1] The efficiency of each phosphate, applied immediately before planting to the limed or unlimed soil, is taken as 100; and the efficiency of each phosphate applied 6 weeks before planting, is expressed comparatively.
[2] Soil No. 213 not included.

Incorporation of the phosphates with the soil six weeks before planting diminished the efficiencies of the five phosphates very appreciably in practically all soils whether lime was used or not.[6] The mean values for the different phosphates show that on the whole acid phosphate possibly lost slightly more of its efficiency than did double superphosphate or basic slag in both the unlimed and the limed series. Although bone meal and floats lost still less than did double superphosphates or basic slag, all these materials had a well defined tendency to be less, rather than more, efficient when they were applied six weeks in advance of planting. This is not in accord with the commonly expressed idea that floats and bone meal may be applied before planting is done because their availabilities increase with time.

On the whole, liming did not appreciably affect the losses in efficiency sustained by the various phosphates when the latter were incorporated with the soil. The average results obtained with all the soils indicate that possibly liming diminished the losses of basic slag and double superphosphates very slightly. The differences between the losses in the limed and the unlimed series in the case of these two phosphates, however, are probably no greater than the experimental error.

From these data it would seem that, in judging whether lime should be applied for the sake of its effect on the phosphates, the effect of lime on the immediate efficiency of the phosphate should be considered chiefly.

These results are not in accord with the statement, which has gained authority from constant repetition, that liming tends to maintain the availabilities of phosphatic fertilizers. The results obtained by Wheeler (*32*) on the after effects of certain phosphates on limed and unlimed soil are to some extent confirmatory of the results reported in this paper. In the field experiments of Wheeler it will be noted that while the total yields of millet and potatoes were far greater on the limed than on the unlimed plots, the increases attributable to the phosphates were much

[6] The results for floats in the limed soils are hardly significant. Liming alone reduced the efficiency of this material to such a low figure that the added effect of remaining in the soil could not be measured with accuracy.

greater in nearly every case on the unlimed plots. The reverse was true, however, in the case of Swedish turnips.

The effect of liming on the loss in efficiency of phosphates evidently varies very considerably according to the character of the soil involved. In some soils, such as that of the Rhode Island Experiment Station, the influence of lime may be so pronounced on certain soil conditions affecting growth of particular crops as to mask entirely the effect of the lime on the efficiencies of the phosphates. This possibly may have been true in the case of soil No. 1529 where the efficiency of acid phosphate was markedly increased by liming.

RATE AT WHICH PHOSPHATES LOSE EFFICIENCY IN THE SOIL

In order to learn whether the losses in efficiency of phosphates remaining six weeks in the soil were about the maximum to be expected, or whether greater losses would have taken place had the phosphates been incorporated with the soil for a period longer than six weeks, supplementary experiments were conducted with four of the soils to secure data on the rate of loss. The experiments were conducted in the same manner as those previously described, except that in some tests 2-gallon glazed pots were used instead of 5-gallon containers. The essential details of the tests are given in Table X.

TABLE X.—*Conditions of Experiments X to XIV, inclusive.*

Experiment No.	Soil No.	Quantity of moisture-free soil per pot.	Optimum water-content of soil.	Sodium nitrate per pot.	Ammonium sulphate per pot.	Potassium sulphate per pot.	Number of plants grown per pot.	Number of days plants grew.
		Kgm	*Per cent.*	*Gm.*	*Gm.*	*Gm.*		
X [1]	213	42.07	18	12.6	9.0	12.0	6	35
XI	1578	17.31	30	3.2	2.3	3.0	20	42
XII	1578	6.21	30	5.6	4.0	5.3	12	33
XIII	1529	19.98	13	4.2	3.0	4.0	16	41
XIV	1524	7.80	25	3.5	2.5	3.3	10	39

[1] In this experiment corn was grown instead of millet.

Table XI gives the results of the experiments.

The results of Experiment X show that, in some soils at least, the loss in efficiency which the phosphates undergo depends to a considerable extent upon the size of the application, the larger application losing much less of its efficiency than the smaller. This was one reason why in the other experiments there were applied quantities near the maximum to which the soil would respond.

As in the previous experiments, the losses in efficiency varied considerably, according to the kind of soil involved. The results on the whole indicated that acid phosphate continued to lose in efficiency the longer it remained in the soil, although the loss was exceedingly small after the first 20 to 30 days. This is illustrated in figure 1, the curve of which is a composite of the curves plotted from the results given in the unlimed series of Table IV, Experiment III, and Table XVI, Experiments X, XI, XII, XIII, and XIV. It does not, therefore, show the rate of loss in any one soil. In this curve the efficiencies of the acid phosphate applied at various lengths of time before planting are plotted relative to 100 for the

efficiency of that which was applied immediately before planting was done.

It should be borne in mind that the losses represented in the curve and in the foregoing tables were in excess of those sustained by acid phosphate applied immediately before planting. Since the plants could not have utilized any appreciable quantities of phosphoric acid during the first 10 to 20 days after they were planted, it is evident that very considerable losses in the efficiencies of the phosphates probably occurred which are not shown in the experimental results. An idea of what these losses were can be obtained by extrapolating the curve beyond its origin to show what the efficiency of acid phosphate would have been had the phosphate been incorporated with the soil the day plants were ready to use it.

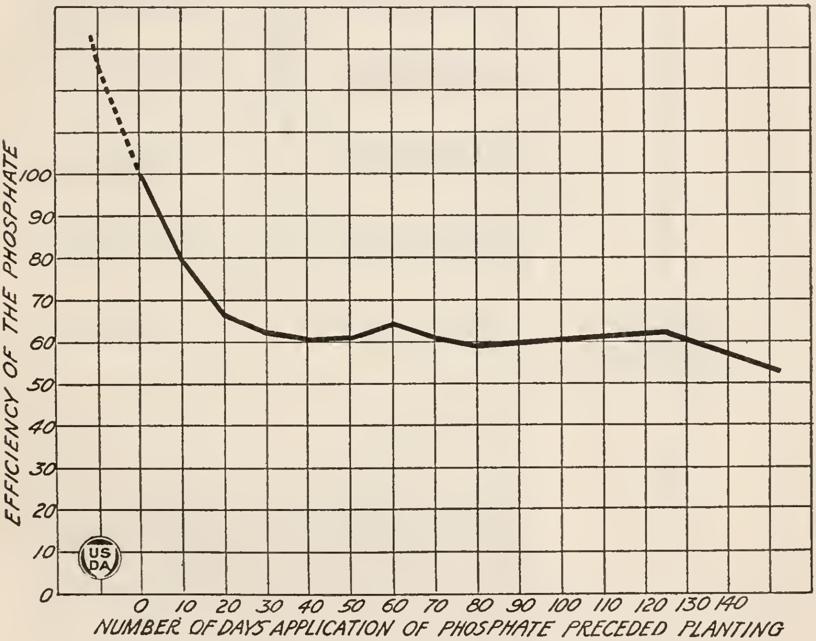

FIG. 1.—Efficiency of acid phosphate as affected by time of application.

These experiments do not show what loss in efficiency might be sustained by the phosphates after they had been used on these soils for a series of years. One is inclined to think that repeated applications of phosphates would bring about such conditions in a soil that succeeding applications would sustain smaller losses in efficiency on remaining in the soil. The results of long-continued plot experiments, however, do not for the most part support this view, since the later applications of phosphatic materials in these experiments do not seem to be relatively more efficient than the earlier ones.

The losses in efficiency established in these experiments for phosphates which remained a short time in unplanted soils are greater than are usually conceived as taking place. In experiments by Schneidewind (*25*, *26*) on a loess soil and a red sandy soil basic slag and acid phosphate apparently lost nothing in efficiency by remaining in the soil. There has been very little experimentation along this line, however. Determina-

TABLE XI.—*Results of experiments conducted with four soils to learn the rate at which acid phosphates and basic slag lose their efficiency*

Experiment	Time phosphate was mixed with the soil.	Kind of phosphate applied.	Quantity of phosphoric acid (P_2O_5) applied per pot.	Green weight of plants (stalks and leaves) in individual pots.						Average weight per pot.	Relative efficiency of the phosphates. (Acid phosphate applied the day of planting=100.)
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
Experiment X. Soil No. 213	Control	None	0	422	116	128	119	142	....	185	....
	Day planting was done	Acid phosphate	.60	493	270	256	197	256	....	294	100
	Do	do	1.20	502	519	558	296	460	....	467	100
	Do	do	2.10	895	880	850	798	857	....	856	100
	Do	do	3.15	927	1,045	980	1,072	....	....	1,006	....
	18 days before planting	do	.60	225	225	281	255	239	....	245	55
	Do	do	2.10	818	480	797	592	737	....	685	81
	39 days before planting	do	.60	232	282	229	210	232	....	237	48
	Do	do	2.10	719	745	643	634	672	....	683	81
	81 days before planting	do	.60	204	257	222	213	228	....	225	38
	Do	do	2.10	634	763	675	578	584	....	647	77
				Oven-dry yield of plants (heads, leaves, and stalks) in individual pots.						Average oven-dry yield.	
Experiment XI. Soil No. 1578	Control	None	0	0.2	0.2	0.3	0.2	....	....	0.2	....
	Day planting was done	Acid phosphate	.4	5.2	5.8	5.3	6.1	....	....	5.6	100
	Do	do	.8	21.0	11.9	14.8	19.8	....	....	16.9	100
	Do	do	1.2	30.3	21.1	23.2	28.0	....	....	25.7	100
	Do	do	2.0	44.7	37.9	40.9	52.6	....	....	44.0	100
	7 days before planting	do	1.2	21.4	18.3	15.4	17.7	....	....	18.2	72
	14 days before planting	do	1.2	15.9	18.5	18.6	15.5	....	....	17.1	68
	28 days before planting	do	1.2	15.9	17.7	13.6	14.0	....	....	15.3	62
	56 days before planting	do	1.2	13.7	8.8	9.6	8.9	....	....	10.3	48

Experiment XII. Soil No. 1578	Control	None	0	.14	.12	.13	.12	0.20	0.15	.14	....
	Day planting was done	Acid phosphate	.15	4.07	3.71	....	3.39	5.97	3.67	4.16	100
	Do	do	.30	5.69	5.37	5.40	4.69	6.66	5.55	5.56	100
	Do	do	.60	7.43	6.37	8.32	6.76	8.68	6.84	7.40	100
	Do	do	.90	7.86	6.67	7.37	8.67	9.83	8.14	....	....
	Do	do	.90	8.68	....	....	....	....	....	8.17	100
	Do	do	1.80	8.45	9.50	8.55	9.95	9.49	10.61	9.43	100
	20 days before planting	do	.90	6.86	6.67	6.77	7.92	8.20	7.24	7.28	64
	40 days before planting	do	.90	6.51	6.61	6.87	7.41	7.04	7.33	6.96	59
	83 days before planting	do	.90	6.44	7.08	6.40	7.18	7.57	7.78	7.08	61
	125 days before planting	do	.90	8.49	6.07	6.94	7.58	6.09	7.73	7.15	62
	167 days before planting	do	.90	6.69	4.23	7.37	5.96	7.14	7.50	6.48	50
Experiment XIII. Soil No. 1529	Day planting was done	None	0	3.9	2.6	3.0	2.2	....	....	2.9	....
	Do	Acid phosphate	.2	11.9	11.0	10.2	9.6	....	....	10.7	100
	Do	do	.4	20.5	18.8	17.1	12.2	18.4	....	17.4	100
	Do	do	.8	25.2	32.6	25.2	28.8	....	....	28.0	100
	Do	Basic slag	.4	28.7	21.2	26.5	21.5	....	....	24.5	168
	14 days before planting	Acid phosphate	.4	16.6	19.7	16.4	15.3	....	....	17.0	96
	Do	Basic slag	.4	22.7	26.6	20.1	20.7	....	....	22.5	149
	28 days before planting	Acid phosphate	.4	13.6	10.2	18.8	10.9	....	....	13.4	70
	Do	Basic slag	.4	16.6	15.5	19.2	17.9	....	....	17.3	99
	56 days before planting	Acid phosphate	.4	14.6	13.6	14.7	13.9	....	....	14.2	76
	Do	Basic slag	.4	21.0	17.3	15.2	15.3	....	....	17.2	99
	88 days before planting	Acid phosphate	.4	9.1	15.6	11.6	10.5	10.8	....	11.5	55
	Do	do	1.2	29.8	28.0	27.5	29.6	26.7	....	28.3	68
Expr. XIV. Soil No. 1524	Control	None	0	.6	.7	.8	.9	....	....	.8	....
	Day planting was done	Acid phosphate	.2	5.5	5.1	4.9	4.1	....	....	4.9	100
	Do	do	.4	9.6	8.5	9.0	8.8	....	....	9.0	100
	Do	do	.6	12.8	10.0	14.1	12.5	....	....	12.4	100
	14 days before planting	do	.4	6.6	6.4	6.9	5.6	....	....	6.4	69
	28 days before planting	do	.4	6.0	5.7	6.0	4.9	....	....	5.7	60
	56 days before planting	do	.4	4.7	4.8	5.6	6.3	....	....	5.4	55

tions that have been made of the "residual" or "after-effects" of phosphatic applications have not been so conducted as to throw much light on this subject.[7]

That such losses in efficiency or in "availability" may be very considerable and very general seems to be indicated by the fact that as a rule only a small portion of the phosphoric acid applied to the soil is recovered in the crop. While recoveries in the crop of 60 to 90 per cent of the nitrogen or potash applied are common, recoveries of phosphoric acid usually range much lower—from 10 to 20 per cent (*15*), or sometimes even 2 per cent. That this low recovery of phosphoric acid is due to interaction between phosphates and certain soil constituents is indicated by the experiments of Pfeiffer and Simmermacher (*20*) in quartz sand.

SUMMARY

(1) The relative efficiencies of acid phosphate, rock phosphate, bone meal, basic slag, and double superphosphate were tested in nine different soils in which millet was grown as the crop. The effect of the length of time the phosphates remained in the soil and the influence of liming on the relative efficiencies of the phosphates were also determined.

(2) The relative efficiencies of all the phosphates varied widely in the different soils. Bone meal and rock phosphate were particularly affected by the character of the soil. In one soil the efficiency of rock phosphate was about the same as that of acid phosphate, while in another soil it was only 4 per cent that of acid phosphate.

(3) None of the phosphates varied in efficiency directly with the lime requirement of the soil, although rock phosphate and bone meal were generally most effective in the soils with high lime requirements.

(4) Applications of lime equivalent to the lime requirement of the soil (determined by the Veitch method) decreased the efficiencies of acid phosphates, basic slag, and double superphosphate in some soils and increased them slightly in others. In two soils liming had little influence on bone meal, but in the seven other soils it markedly decreased the efficiency of the bone meal. The efficiency of rock phosphate was decreased by liming to an approximately constant value in all soils—about 3 per cent that of acid phosphate.

(5) Practically no difference in efficiency was observed regardless of whether the lime was applied to the soil six weeks before or immediately before the phosphate was applied.

(6) A considerable further decrease in the efficiency of bone meal occurred when the quantity of lime applied was increased beyond the amount indicated by the lime requirement of the soil.

(7) It is probable that even in those soils where the efficiencies of acid phosphate, basic slag, and double superphosphate were increased by the quantity of lime applied a larger application of lime would have decreased the efficiencies of these materials.

(8) A comparison was made of the efficiencies of the phosphates applied six weeks before planting with the efficiencies of the materials applied immediately before planting. When the phosphates remained six weeks in the soil the efficiencies of the five phosphates diminished very appreciably in all soils whether limed or not. The losses in efficiency attributable to the phosphates remaining in the soil were greater

[7] This is chiefly due to the fact that the crop residues have not been removed before the second crop was planted, and it is therefore impossible to ascertain how much of the phosphoric acid assimilated by the second crop was secured from the decomposition of the organic residues of the first crop and how much from the unassimilated phosphoric acid left in the soil.

in the cases of acid phosphate, basic slag, and double superphosphate than in the case of bone meal or rock phosphate.

(9) Acid phosphate continued to lose in efficiency the longer it remained in the soil, although after the first 20 to 30 days the loss was exceedingly small. That such losses in efficiency are of general occurrence and that they are due to the action of soil constituents rendering the phosphoric acid unavailable to the plant is indicated by the fact that, as a rule, only 10 to 20 per cent of the phosphoric acid applied is recovered in the crop, whereas 60 to 90 per cent of the nitrogen or potash applied is commonly recovered.

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GROWTH OF FRUITING PARTS IN COTTON PLANTS[1]

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INTRODUCTION

The rate of development of the cotton plant and especially of the fruiting branches, deserves more consideration in connection with studies of cultural methods and weevil-control problems.

During the past three years data have been recorded on the order and rate of appearance and growth of floral buds, the sequence of flowers, and the growth of bolls. These studies have been carried on under different conditions and upon different types of cotton, as indicated in the following outline:

1. Grown under the dry atmospheric conditions of the irrigated valleys of Arizona: Pima variety of the Egyptian type, Upland varieties of Lone Star, Acala, and Durango.
2. Grown under drought conditions on the "Black Land" belt near Greenville, Tex.: Lone Star.
3. Grown under conditions of high humidity at James Island near Charleston, S. C.: Sea Island and Meade.

Thus it is possible to make comparisons of corresponding phases of plant growth and development with different types of cotton under a wide range of environmental conditions.

The records summarized in the following tables show a very close agreement in the rate of appearance of floral buds and blooms between distinct species and types of cotton grown under different conditions. Considerable variation was observed between varieties in the period of development of the floral bud and in the interval from date of flowering to boll maturation. This indicates the importance of considering the relation of varietal and environmental factors to the growth rate.

PRODUCTION OF THE FRUITING BRANCHES

The main stalk of the cotton plant is formed by the development of successive internodes. At each node two buds are normally developed, an axillary bud which produces the vegetative branch and an extra-axillary bud which produces the fruiting branch. The axillary bud stands just above the middle of the base of the subtending leaf and usually remains dormant except on the first few nodes at the base of the stalk. The extra-axillary bud is developed to the right or left of the axillary bud. The specialized nature of the axillary and extra-axillary buds and the tendency for each to develop on definite groups of nodes

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on the main stalk has been recognized and described in several publications.[2]

The rate at which fruiting branches are formed on the main stalk is an important factor in the production of fruit. Data on formation of branches have been obtained in the United States from several varieties of cotton, at Sacaton, Ariz., Greenville, Tex., and Charleston, S. C. The data from Sacaton and Charleston show the intervals between the first appearance of floral buds on successive branches, while those from Greenville show the intervals between the flowers. Harland [3] working with Sea Island cotton in the West Indies determined the rate of appearance of fruiting branches by using the interval between flowers on the first nodes of successive branches.

The first indication of a new fruiting branch is the appearance of a minute triangular bud, commonly called a "square," deeply inclosed between the stipules of the primary leaf. The appearance of the square always precedes the development of the internode on which it is borne, and may therefore be considered as a definite indication of the formation of a fruiting branch. Table I gives the mean interval between the appearance of successive fruiting branches for each variety. It is evident that there is a close agreement of varieties in the rate of production of fruiting branches.

TABLE I.—*The rate of formation of fruiting branches of different types of cotton*

Variety.	Locality.	Year.	Average number of days between the appearance of successive fruiting branches.
Lone Star	Sacaton, Ariz	1921	3.30±0.088
Acala	....do	1921	2.80± .067
Durango	....do	1921	2.87± .097
Pima Egyptian	....do	1921	2.81± .043
Lone Star [1]	Greenville, Tex	1922	2.36± .038
Meade	Charleston, S. C	1922	3.03± .156
Sea Island	....do	1922	2.86± .081

[1] Figured from number of days between flowering dates.

It should be stated that these data represent the mean number of days between the appearance of successive fruiting branches for the entire period of observation. A comparatively wide range in the interval between the appearance of branches was found, but no significant difference could be traced for different periods of growth or groups of nodes. Individual records of the number of days between the appearance of branches ranged from one to six days, but these differences occurred at no definite period.

[2] COOK, O. F. DIMORPHIC BRANCHES IN TROPICAL CROP PLANTS. U. S. Dept. Agr. Bur. Plant Indus. Bul. 198, 64 p., 9 fig., 7 pl. 1911.
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MCLACHLAN, Argyle. THE BRANCHING HABITS OF EGYPTIAN COTTON. U. S. Dept. Agr. Bur. Plant Indus. Bul. 249, 28 p., 3 pl. 1912.

[3] HARLAND, S. C. MANURIAL EXPERIMENTS WITH SEA ISLAND COTTON IN ST. VINCENT, WITH SOME NOTES ON FACTORS AFFECTING THE YIELD. *In* West Indian Bul., v. 16, p. 183. 1917.

The data from Sacaton and Charleston were obtained under conditions of unchecked growth, and no seasonal variation in the rate of branch formation was shown in the records. At Greenville, however, the development of the plants was stopped by a drought in July and August. The checking effect of this drought was apparent late in July, and all squares produced after that period were shed before the flowering stage was reached. Yet the interval between first blooms of successive branches at the end of the flowering period was not consistently longer than the interval between blooms early in the season. This indicates that a reduced rate of growth of the plants did not materially influence the rate of appearance of fruiting branches during the flowering period. It is possible, however, that if the subsequent squares had reached the flowering stage, a definite increase in the interval between blooms might have been found.

The data on Lone Star grown at Sacaton, Ariz., and Greenville, Tex., show the widest difference in the mean interval between the appearance of fruiting branches. The data on the other varieties show a close agreement in the rate of production of fruiting branches. That such uniformity in rate of production could be obtained under widely different conditions of soil, climate, and moisture seems remarkable.

GROWTH OF FRUITING BRANCHES

The fruiting branch is formed by the development of a series of joints, or internodes, each bearing a floral bud, or "square." As described previously in this paper, the first indication of the fruiting branch is the appearance of a minute square and its subtending leaf, inclosed between the stipules of the leaf on the axis. Following the appearance of the square, the first internode of the fruiting branch begins to lengthen, carrying the square and its leaf away from the main stalk. As growth proceeds in the first internode of the fruiting branch, the bud which will form the second node slowly develops until the second square and subtending leaf may be seen. This procedure is followed throughout the growth of the fruiting branch, the preceding node growing for a certain interval before the next square appears.

Different types and varieties of cotton differ in the length and the number of internodes of the fruiting branch. The rate of production of squares, however, is the factor of greatest importance to be considered, particularly under boll-weevil conditions.

In the season of 1921 at Sacaton, Ariz., the date of appearance of each square on each branch was recorded on 10 plants each of the following varieties: Lone Star, Durango, Acala, and Pima Egyptian. From these records it is possible to find the average number of days between the appearance of successive squares on any branch or at any group of internodes.

A similar series of records was obtained in 1922 on the Sea Island and Meade varieties of cotton grown at Charleston, S. C. Data were obtained on the Lone Star variety at Greenville, Tex., the interval in this case being determined by the number of days between blooms on successive internodes.

These data, presented first in Table II, show an average of about six days between the appearance of successive squares for all the fruiting branch internodes of the plant. It will be noted that the production of new squares on the fruiting branches proceeds at Sacaton at the same

rate, regardless of the variety, although they represent widely different types.

While the Meade and Sea Island are distinctly different types of cotton and were grown under conditions where rainfall is frequent and a relatively high percentage of humidity obtains, the interval between the appearance of the square remains the same as with other varieties grown under irrigation in the Southwest.

It will be noted that the mean number of days between the appearance of squares on fruiting branches of Lone Star grown at Sacaton, Ariz., is about one day more than for the same variety grown in Texas. The fact that appearance of squares was used at one place while date of flowering was used at the other could hardly explain this difference. It is possible that the short flowering period at Greenville, caused by drought, may have resulted in a lower mean interval than would have resulted from data obtained over a longer period.

The records obtained at Greenville represent data from flowers on fruiting branches developing from the seventh to the thirteenth node of the main stalk. At Sacaton, however, the data on appearance of squares were obtained from branches developing from the twelfth to the twenty-first node. The difference in the interval between squares or blooms at the two places, if significant, should probably be attributed to the fact that data were obtained at different stages of plant development.

RATE OF APPEARANCE OF SQUARES IN RELATION TO CLOSE SPACING OF PLANTS

The fact that first squares on successive fruiting branches are produced more rapidly than successive squares on the fruiting branches, is of practical significance in connection with the cultural advantages that have been obtained by the use of closer spacing of plants in the row. As the first squares on successive fruiting branches appear at about three-day intervals, while successive squares on the fruiting branches appear at about six-day intervals, a more rapid setting of fruit would be expected if the number of plants was increased. With a larger number of branches, resulting from more plants to the acre, better advantage is taken of the more rapid production of squares on the first node of successive fruiting branches.

TABLE II.—*The rate of appearance of successive squares on fruiting branches*

Variety.	Locality.	Year.	Mean number of days between the appearance of successive squares on the fruiting branch.
Lone Star	Sacaton, Ariz	1921	6.60±0.213
Acala	.....do	1921	6.10± .160
Durango	.....do	1921	6.30± .192
Pima-Egyptian	.....do	1921	6.36± .069
Lone Star [1]	Greenville, Tex	1922	5.65± .068
Meade	Charleston, S. C	1922	6.0 ± .064
Sea Island	.....do	1922	6.1 ± .113

[1] Figured from number of days between flowering dates.

LATE SEASON INCREASE IN INTERVAL BETWEEN SQUARES

There is a tendency for the interval between the appearance of squares to lengthen as the season advances. This is shown in Table III which gives the number of days between the appearance of successive squares on 10 Pima Egyptian plants for three 3-week intervals. In 1921 the mean period for the squares produced from June 15 to July 5 was 5.36 days, from July 6 to July 26 it was 6.45 days, and from July 27 to August 15 it was 7.53 days. It can be seen that the mean period for each successive three weeks was longer. These data are substantiated by figures on Pima at Sacaton in 1920, which showed that the period between the opening dates of flowers on successive internodes was gradually increasing. This also is shown in Table III. It will be noted that the increase is greater in the 1920 data, but this may be ascribed to the fact that the period from the appearance of a square until it flowers is slightly increased as the season advances, thus tending to make the interval between successive flowering dates slightly greater than the interval between the appearance of successive squares.

TABLE III.—*Mean interval between the appearance of successive squares or flowers on fruiting branches for three consecutive 3-week periods, at Sacaton, Ariz.*

	Period I, June 15 to July 5.	Period II, July 6 to July 26.	Period III, July 27 to Aug. 15.
	Days.	*Days.*	*Days.*
Interval between squares on Pima, 1921...	5.36±0.113	6.45±0.115	7.53±0.132
Interval between flowers on Pima,[1] 1920...	5.91± .068	7.14± .098	8.85± .231

[1] The intervals are based on flowering periods 30 days later than the square periods used the following year. This represents the time from appearance of square to bloom, and places the two series on a comparable basis.

The lengthening of the period between the production of new squares has just been shown to be correlated with the advance of the season. The possibility that the period lengthens because the new internodes are farther out on the fruiting branch naturally suggests itself. The interval between the appearance of the first square on a fruiting branch and the appearance of the second was figured for the 10 Pima plants in 1921. This interval was compared with the interval from the appearance of the second square until the third square appeared, and so with other joints, along the fruiting branches. A summary of these data is given in Table IV.

It will be noticed that the period does increase progressively between the successive internodes of the branches. The difference, however, is slight and it is believed that the reason for the increase is that these nodes were produced later in the season, which, as has been shown in Table III, results in a longer interval. Such differences, however, were not found to be correlated with the node numbers representing their positions on the branches.

When comparisons were made of the interval between the appearance of squares formed during the same three-week period on the first and second nodes and on the fourth and fifth nodes of fruiting branches, it was found that the interval between squares on the outer nodes of lower fruiting branches was practically the same as the interval

between squares on the first and second nodes of branches farther up on the main stalk. The mean interval between the appearance of squares on the fourth and fifth nodes was 6.2 days, while the mean interval between the appearance of squares on the first and second nodes was 6.05 days, for squares appearing within the three-week period of July 6 to July 26.

TABLE IV.—*Average interval between the successive appearance of squares for each internode on 10 Pima plants, Sacaton, Ariz., 1922*

Internodes.	Interval.
	Days.
First to second	5.65
Second to third	6.01
Third to fourth	7.39
Fourth to fifth	6.82

DEVELOPMENT PERIOD OF THE FLORAL BUD OR "SQUARE"

The number of days from the appearance of a square[4] until it flowers is a feature in the growth of cotton that has received little attention by investigators, although it is of considerable importance in the analysis of late or early varieties and in relation to weevil damage.

In the season of 1921, the "square period"—as the interval between the appearance of the square and the date of flowering has been termed—was recorded for several varieties of cotton at Sacaton, Ariz. The information was obtained on three Upland varieties for squares that appeared between June 15 and July 10 and for squares of Pima cotton between June 15 and August 15. (See Plate 1, showing the early development of Pima floral buds.) These data are presented in Table V, giving the mean square period as obtained from the above data. It will be noticed that the mean period for Pima was 30.11 days, while that of the three Upland varieties was about 23 days, a difference of approximately 7 days. From similar data obtained on James Island, near Charleston, S. C., the mean square period for Sea Island was found to be 33.06 days and for Meade cotton 28.45 days. Such data illustrate in a practical way one of the principal reasons why the Sea Island and Egyptian types of cotton are later than the Upland varieties.

TABLE V.—*Interval from appearance of squares until flowering date*

Variety.	Locality.	Year.	Mean square period.
			Days.
Lone Star	Sacaton, Ariz	1921	23.20±0.337
Acala	....do	1921	22.80± .230
Durango	....do	1921	23.40± .232
Pima Egyptian	....do	1921	30.11± .128
Sea Island	South Carolina	1922	33.04± .127
Meade	....do	1922	28.45± .104

[4] The term "appearance of a square" is used in this paper to designate the time that the three bracts of a fruiting bud become visible to the naked eye as a minute triangular form, approximately one-thirty-second inch in diameter.

In order to see if there was a lengthening of the square period as the season advanced, the data relating to the Pima variety were divided and compared in three successive three-week intervals. As shown in Table VI, the mean square period from June 15 to July 5 was 28.32 days, from July 6 to July 26, 31.11 days, and from July 27 to August 15, 31.43 days. From these data it appears that the square period is shorter early in the season than later, but that the increase of the third three-week interval is not significantly greater than that of the second three-week interval. This relation could not be determined in the other varieties, since the data were not recorded for sufficiently long intervals.

TABLE VI.—*Mean square period for three 3-week interval of Pima at Sacaton, Ariz., 1921*

	Squaring period.		
	Period I, June 15 to July 5.	Period II, July 6 to July 26.	Period III, July 27 to Aug. 15.
Mean number of days	28. 32±0. 115	31. 11±0. 067	31. 43±0. 115

A further comparison of the mean period required for the development of squares on the successive internodes of the fruiting branches was made on the Pima at Sacaton in 1921. These data are summarized and presented in Table VII. The mean square period at each node is seen to increase slightly after the second node. This increase, however, is regarded as being due to the fact that the squares on the outer internodes were produced later, and it has been previously shown that the later squares have a longer square period regardless of their position on the fruiting branches. From these data there is no significant evidence that the square period is longer because the square is produced at the outer nodes, toward the end of the branch. This is evident when the periods for squares of first nodes are compared with periods for squares of fifth nodes that appeared on the same dates.

TABLE VII.—*Mean square period for the successive internodes of the fruiting branches on Pima, Sacaton, Ariz., 1921*

	Fruiting branch internode No.—				
	1	2	3	4	5
Mean number of days	29. 4	29. 4	30. 1	30. 7	31. 1

GROWTH OF THE FLORAL BUD

A daily record of the size of floral buds was made on the Lone Star variety at Greenville, Tex., in 1922. The size of the bud was determined by depressing one bract of the young square and measuring the length of the bud from the base of the calyx to the tip of the corolla. Measurements were started when the bracts of the square were about

10 mm. in length. The length of the floral bud in these young squares varied only slightly, the average length being about 6 mm. Daily measurements were taken during the development of the bud until it flowered. The interval between the time when the bud was 6 mm. in length and the time when it flowered was found to be about 15 days. No case was found in which this interval exceeded 15 days and only 3 buds out of 24 flowered in a shorter interval.

The mean length of floral buds was computed from the daily measurements of all buds. From these data, which are presented in Table VIII, the mean daily length of the bud may be determined for a period of 15 days preceding bloom. From the differences between the lengths of the bud on successive days, the average daily growth rate can be computed. From the fifteenth to the tenth days preceding bloom, the average daily growth of the bud is approximately 0.5 mm. After the tenth day, the daily growth rate increases slowly until the fourth day before the opening of the flower, at which time the mean length of the bud was 14.28 mm. Growth is rapid thereafter. The mean length of the bud on the day preceding bloom was 24.37 mm., representing a total increase in length of the bud of about 10 mm. during the last 3 days before bloom. The rapid growth of the bud during the last few days is due to the enlargement of the corolla and the inclosed staminal column.

The size of the floral bud is of importance in connection with studies of boll-weevil infestation. It is believed that the buds are not large enough to breed weevils until they have attained a length of about 6 mm. Squares with buds smaller than this have the bracts closely appressed and seldom are entered by the weevils. Also, the buds are too small for the full development of weevil larvae.

Although records were not kept on the date of appearance of squares, it is believed that the first stage recorded in Table VIII represents about the tenth day after the appearance would have been recorded. On this basis the first 10 or 12 days of square growth may be considered as affording no opportunity for the development of a new generation of weevils. Following this period, the square develops through a period of 15 days, during which time it is of sufficient size to permit a larva to develop.

TABLE VIII.—*Growth of floral bud of the Lone Star variety shown by the daily increase in length, Greenville, Tex., 1922*

Number of days preceding bloom.	Mean length of floral bud.	Number of days preceding bloom.	Mean length of floral bud.
	Mm.		*Mm.*
15	6.02	7	11.17
14	6.57	6	12.22
13	7.2	5	13.1
12	7.62	4	14.28
11	8.36	3	15.6
10	8.96	2	17.7
9	9.67	1	24.37
8	10.3		

GROWTH OF LONE STAR BOLLS IN TEXAS

Two series of measurements of the growth of bolls were obtained on the Lone Star variety near Greenville, Tex., 1922, one series representing the growth of bolls at the beginning of the flowering season, the other of bolls produced from later flowers, the last that were able to set bolls under the local conditions of drought. Measurements of boll length were recorded the second day after the bloom, and continued daily until it was certain that the maximum length had been attained. The young bolls at 2 days of age were about 16 mm. long, increasing in length approximately 2 mm. per day thereafter until they were about 12 days old, or about 37 mm. long. After reaching this age the growth rate was much slower, the average maximum boll length of about 41 mm. having been recorded 8 days later, or 20 days after the date of flowering. Table IX presents the mean daily length of these bolls.

It is interesting to note that no increase in length was found after the twentieth day from the bloom. Many bolls reached their full length in 16 days, the mean period from bloom to maximum length being 17.3 days in the first series of bolls and 17.1 days in the second. Of course, these records should not be taken to indicate that development of the boll is complete when the full length is reached, since 17 days represent less than half of the period from the bloom to the open boll.

TABLE IX.—*Daily increase in length of Lone Star bolls, Greenville, Tex., 1922*

Number of days after flowering.	Length of bolls from blooms open July 21 to July 26.	Length of bolls from blooms open Aug. 3 to Aug. 8.
	Mm.	*Mm.*
2	17.6	14.77
3	19.77	16.77
4	21.35	18.18
5	23.55	19.4
6	25.67	20.77
7	28.62	22.2
8	30.02	24.73
9	32.27	26.45
10	33.82	28.55
11	36.1	30.95
12	37.9	34.16
13	39.05	35.68
14	40.35	36.68
15	41.15	37.5
16	41.85	38.27
17	42.25	38.86
18	42.67	39.13
19	43.15	39.27
20	43.17	39.36

MATURATION PERIOD OF LONE STAR BOLLS

It will be noted in Table IX that the later bolls are consistently smaller than the early ones. This shows the inhibiting effect of the drought which checked the growth of plants after the first week in August. The smaller size of the bolls, however, did not result in a shorter period of maturation. Data on the number of days from bloom to open boll,

show that the bolls produced during the late flowering period were actually slower in maturing than the early bolls. The mean number of days between bloom and open boll for flowers opening from July 21 to July 26 was found to be 42.57 days, while that for bolls set from August 3 to August 8 was 44.55 days. A probable error of ± 0.068 days and ± 0.23 days, respectively, was obtained on these means. These determinations show that the increase of 1.98 days in the maturation period of the later bolls is eight times the probable error of the difference, indicating that it is significant.

No rain occurred throughout the maturation period of either the early or late bolls, and the increase of about two days in the period of maturation of the late bolls may have been due to the checking of plant growth by drought. This suggests that boll opening was not due primarily to the effect of atmospheric conditions in drying the bolls. Excessive shade or moisture, or lower temperature, undoubtedly tends to defer boll opening, but under consistently dry conditions a longer interval between bloom and open boll in later bolls may be due to a retarding of growth processes. It might be expected that drought would result in a premature opening of bolls, but the results obtained at Greenville did not indicate any such effect. It is probable, however, that more severe conditions would result in premature opening.

GROWTH OF PIMA BOLLS IN ARIZONA

Other data on the growth of bolls were obtained from the Pima Egyptian variety at Sacaton, Ariz., in 1921. These records include determinations of the volume, green weight, and dry weight of the growing bolls at regular intervals after flowering.

Table X presents the average volume and weight of Pima bolls as determined from 60 boll samples collected at five-day intervals. From this table it can be seen that the average Pima boll grows very rapidly, reaching its mean maximum volume of 14 cc. at the age of 25 days. Even at 15 days the bolls are nearly full size. (Pl. 2.) The growth, however, as shown by green weight is not so rapid, the mean maximum of 13.4 gm. being obtained at the age of 40 days. Measured by dry weight, the growth is even less rapid, and the mean maximum of 3.8 gm. is not reached until 50 days of age.

It is evident from these data that the average Pima boll reaches its maximum volume in less than half of the period between the dates of flowering and opening of the boll. The green weight of bolls attains a maximum and then declines before maturity is reached. Growth by dry weight gives the best index of progress toward mature development, the maximum of dry weight of the average boll being attained at least 10 days before opening.

TABLE X.—*Volume, green weight, and dry weight per boll at 5-day intervals of Pima cotton at Sacaton, Ariz., 1921, based on the average of 60 bolls of each age that flowered in August*

Age of boll.	Volume.	Green weight.	Dry weight.
Days.	*Cc.*	*Gm.*	*Gm.*
5	.87	.72	.12
10	3.14	2.96	.45
15	8.91	7.93	1.26
20	12.59	11.02	1.99
25	14.66	12.86	2.56
30	14.60	12.93	2.82
35	14.65	13.03	3.12
40	14.90	13.45	3.36
45	14.80	13.15	3.76
50	14.65	13.16	3.98
55	14.10	12.14	3.99
60	....	10.30	3.83
65	....	[1] 4.53	3.99

[1] Open bolls.

MATURATION PERIOD OF PIMA BOLLS

A range in the period of maturation from 45 days to 80 days was obtained on normal Pima bolls in this study, with the period lengthening for the bolls of later flowering dates. This is in agreement with results reported by King,[5] who found that the period of maturation of Pima bolls in Phoenix, Ariz., varied from 54 days for those bolls set in July to 82 days for those set in September. Sufficient data were secured in 1921 on bolls of different flowering dates to indicate that at least three factors are involved in the lengthening of the period of maturation of Pima bolls at Sacaton. First, the early bolls were smaller than those set later in the season; second, the early bolls attained full structural development in fewer days than those set later; and third, the early bolls showed a more rapid reduction of the boll moisture after reaching mature structural development, so that the opening stage was reached in fewer days.

GROWTH OF SEA ISLAND AND MEADE COTTON BOLLS IN SOUTH CAROLINA

The growth of Sea Island and Meade bolls near Charleston, S. C., in 1922, is shown in Table XI, which gives the mean volume and weight per boll of 50 boll samples collected at seven-day intervals. It can be readily seen from this table that the bolls grow very rapidly to full size, as in the other experiments. The Sea Island bolls reach their mean maximum volume of 19 cc. at 21 days of age, and the Meade bolls also attained their larger volume of 29 cc. in the same number of days.

In comparing the time required for bolls to reach full size, these data are only slightly different from those obtained with the Lone Star variety at Greenville, where the full size was reached in about 20 days. There is more contrast with the data secured from the Pima cotton at Sacaton, where the mean maximum volume was found to be reached at the age of 25 days.

[5] KING, C. J. WATER-STRESS BEHAVIOR OF PIMA COTTON IN ARIZONA. U. S. Dept. Agr. Bul. 1018, 24 p., 3 fig., 4 pl. 1922. Literature cited, p. 23–24.

TABLE XI.—*Volume and green weight per boll at seven-day intervals of Sea Island and Meade cotton, near Charleston, S. C., 1922, based on the average of 50 bolls collected at each age*

Age of boll.	Sea Island.		Meade.	
	Volume.	Green weight.	Volume.	Green weight.
Days.	*Cc.*	*Gm.*	*Cc.*	*Gm.*
7	2.40	2.24	4.78	4.26
14	13.13	10.91	21.00	18.23
21	19.95	16.16	29.22	26.64
28	18.50	15.65	30.30	27.53
35	19.63	15.81	30.63	27.88
42	19.61	15.88	29.53	26.65
49	19.00	15.23	....	22.70
56	....	[1] 6.79	....	....
63	....	[1] 5.70	....	....

[1] Open bolls.

MATURATION PERIOD OF SEA ISLAND AND MEADE BOLLS

A mean maturation period of 57.6 ±0.013 days was obtained for Sea Island bolls near Charleston in 1922, from data recorded from bloom to open boll on 988 bolls. The period of maturation showed a definite increase as the season advanced. A mean period of 56.9 days was obtained from bolls set from flowers blooming between June 22 and 28, while a mean period of 62.6 days was found for those set from flowers blooming between August 3 and August 6. The probable errors for these periods were ±0.23 days and ±0.60 days, respectively, showing that the increase in the period of maturation is significant and not due to chance.

The mean period of maturation for the Meade variety at Charleston in 1922 was 56.14 days, as determined from 277 bolls, with a probable error of ±0.11 days. A tendency for the period of maturation to increase as the season advanced was noted, but the data are not presented on account of the small number of bolls and the shortness of the period during which they were set. It is of interest to note that there is a difference of only one day between the mean maturation period of Sea Island and Meade bolls in this location. In other words, the Sea Island bolls require, on the average, about one day longer to open than the Meade bolls.

SUMMARY

(1) Data relating to the growth of the cotton plant are given, including the rate of floral bud production, the period of development from the appearance of a floral bud to flower, and the growth of the boll from flower to maturity. This information is needed in connection with cultural methods and weevil control problems.

(2) A comparison of similar phases of plant growth and development was obtained on several varieties under widely different environmental conditions—namely, Lone Star, Acala, Durango, and Pima Egyptian, at Sacaton, Ariz., in 1921 and 1922; Lone Star, near Greenville, Tex., in 1922; and Meade and Sea Island near Charleston, S. C., in 1922.

(3) The average number of days between the production of successive fruiting branches was approximately three days, with none of the varieties showing significant differences. (See Table I.)

(4) All the varieties, under the conditions represented, showed an average of about six days between the appearance of successive squares on the fruiting branch. (See Table II.)

(5) The Pima variety in Arizona showed a tendency for the interval between the appearance of successive squares of the fruiting branches to lengthen as the season advanced. (See Table III.)

(6) A comparison of the interval between the appearance of successive squares on the fruiting branches showed that the interval increases progressively along the branch. This increase may be due to the fact that outer squares are produced later in the season when growth is slower. The intervals between squares that are produced at the same dates are approximately equal regardless of the positions of the squares on the branches. (See Table IV.)

(7) The interval between appearance of a square and its date of flowering, termed the "square period," showed consistent differences between varieties. The square period for Sea Island was approximately 33 days, for Pima 30 days, for Meade 28 days, and for Lone Star, Acala, and Durango 23 days. (See Table V.)

(8) A tendency for the square period to lengthen as the season advanced was noted on the Pima variety at Sacaton. (See Table VI.) This relation could not be determined in the other varieties since the data were not recorded for sufficiently long intervals.

(9) A slight increase in the square period for each successive node of the fruiting branch was also found on the Pima cotton at Sacaton, but this is probably due to the squares being produced later in the season, and is not correlated with position on the branch. (See Table VII.)

(10) Growth rates of floral buds of Lone Star cotton at Greenville, Tex., showed that the buds grew at a nearly constant daily rate until within about 3 days of flowering, when a more rapid growth rate was recorded.

(11) The sizes of the floral buds at different ages indicate that they were not large enough to successfully develop weevil larvae until about 15 days before flowering, or approximately 10 days after the squares appear.

(12) The growth of Lone Star bolls in Texas was very rapid, the mean maximum length of 41 mm. being reached about 20 days after flowering. The early bolls were found to be larger, the later bolls being produced during a drought. Although smaller, the late bolls had a longer maturation period, 44.55 days, while the large early bolls showed a mean maturation period of 42.57 days, or about 2 days less than the late bolls.

(13) Data on the growth of Pima bolls in Arizona were determined by records of volume, green weight, and dry weight of growing bolls, at regular intervals after flowering. The results show that the mean maximum volume per boll, 14 cc., was attained in 25 days after flowering; that the mean maximum green weight per boll, 13.4 gm., was attained in about 40 days; and that the mean maximum dry weight per boll, 3.8 gm., was attained in about 50 days.

(14) A range in the period of maturation from 45 days to 80 days was observed on normal Pima bolls in Arizona in 1921. The period of maturation was found to lengthen for bolls of later flowering dates.

(15) Three factors seemed to influence the lengthening of the maturation period for Pima bolls in 1921: The early bolls were smaller; they reached mature structural development in fewer days; and they reduced boll moisture to the opening stage more rapidly than the later bolls.

(16) The growth of Sea Island and Meade cotton bolls was determined in South Carolina during 1922 by recording the volume and green weight of growing bolls collected at seven-day intervals after flowering.

(17) The Sea Island bolls reached their mean maximum volume of about 19 cc. and their mean maximum green weight of about 16 gm. in 21 days after flowering.

(18) The Meade bolls reached their mean maximum volume of about 29 cc. in 21 days and their mean maximum green weight of about 27 gm. in 28 days after flowering.

(19) A mean maturation period of 57.6 days was obtained for the Sea Island bolls, in comparison with 56.14 days for the Meade bolls, and the period of maturation was found to increase as the season advanced.

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PLATE I

Floral buds of Pima (Egyptian) cotton at Sacaton, Ariz., showing the daily increase in size of the involucre during the early stages of growth, for 10 successive days, from the second to the eleventh day from the "appearance" of the "square." Natural size. Photographed by H. F. Loomis.

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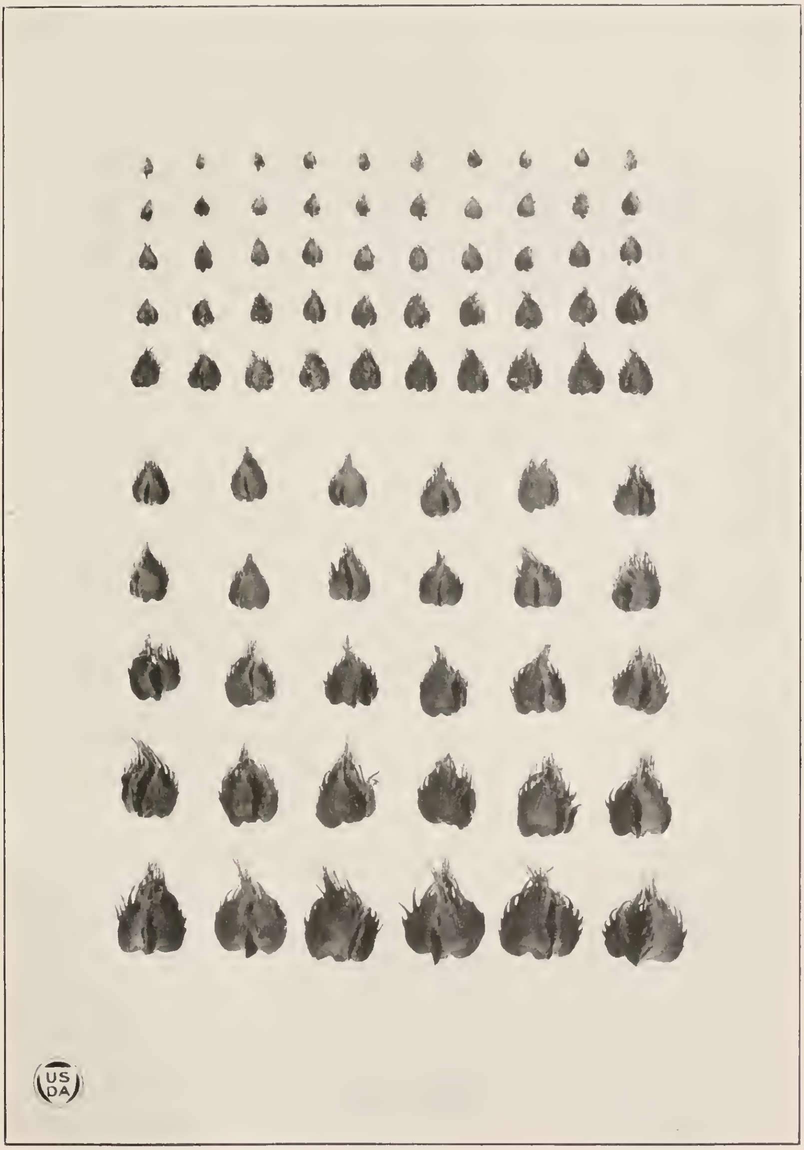

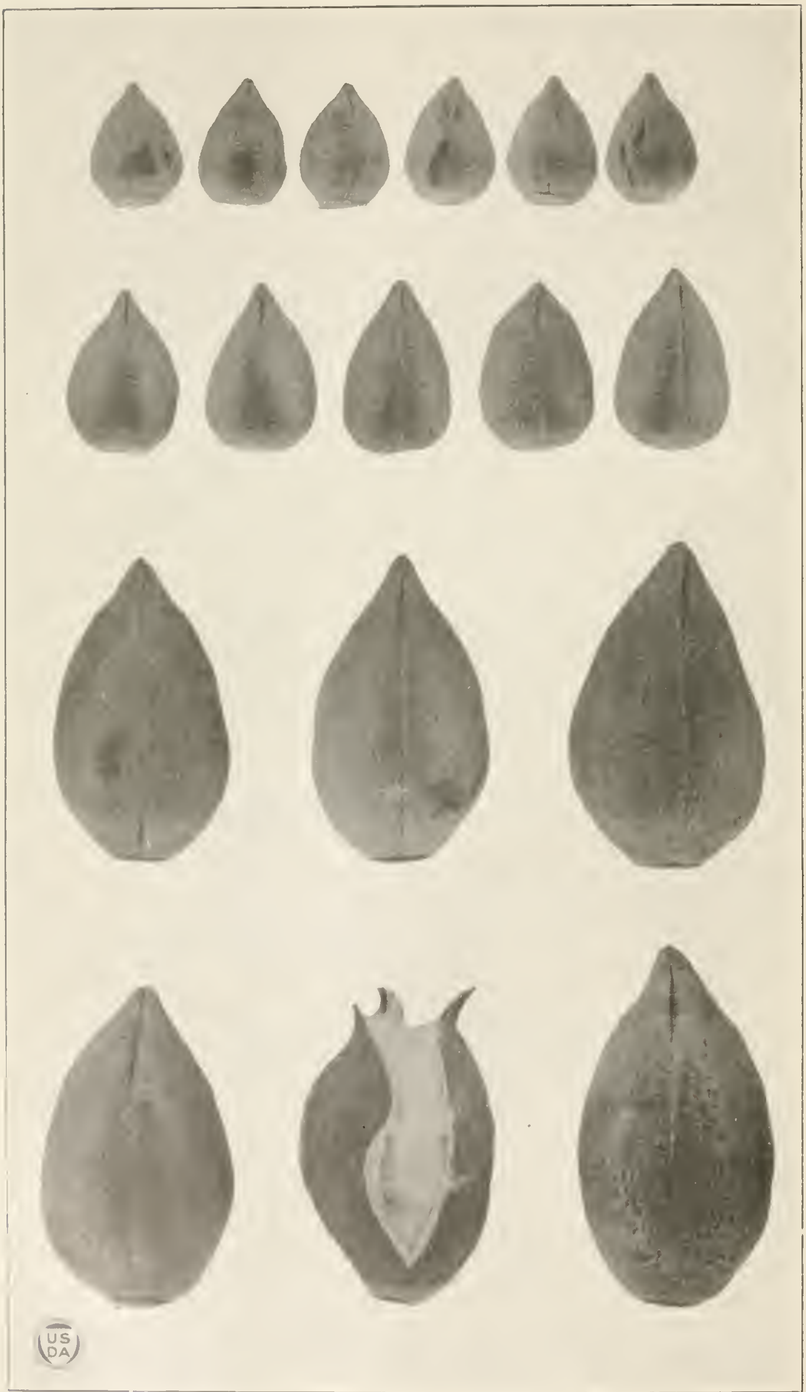

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PLATE 2

Bolls of Pima (Egyptian) cotton at Sacaton, Ariz., showing the size at different stages of development. Beginning at the top row, the ages are 5, 10, 15, and 45 days, respectively, as measured from the flowering date. Natural size. Photographed by R. D. Martin.

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SYSTEMIC INFECTIONS OF RUBUS WITH THE ORANGE-RUSTS[1]

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INTRODUCTION

Cultivation of some of the most desirable varieties of blackberries and raspberries has been limited or discontinued in certain regions because of the orange-rusts which inhibit the development of fruit on the canes infected. Local infection of leaves of blackberries by sowing aecidiospores of the long-cycled orange-rust, Gymnoconia, has been brought about by a number of persons. Reference to such work as has been done along this line has been made by Clinton (*3, 4*)[2] and Kunkel (*7*).

The problem of working out practical methods for the control of these rusts, however, depends upon a knowledge of when and how the gametophytic mycelium enters the host and becomes established as a constitutional parasite. The results of infection experiments dealing with this problem are reported at this time.

The common blackberry has perennial roots from which canes arise year by year, those of one year bearing fruit the next. The turions appear in the spring from the buried crown or from root runners and become the "old canes" of the next year, dying after fruiting. The new canes are of the type having indefinite growth; therefore the formation of a terminal bud which would remain dormant during the winter and push out into new growth the following spring would be very unusual and abnormal, although lateral buds at the lower part of the old cane frequently develop into new branches. The crown usually lies a few inches beneath the surface of the soil, and consequently there is a part of the original cane which lives several years. As new shoots and roots arise from this structure it loses its identity. Nurserymen would refer to that part of the cane beneath the soil simply as the crown because new canes arise in this region. It may also be referred to as the perennial base. Figure 1 shows these features diagrammatically for a simple plant (see also fig. 5). The structure of the cane and the relation of its tissues as they appear in cross-sections are brought out in Plate 2, B.

HOST PARASITE RELATIONS

In dealing with a rust which is perennial and systemic in a woody plant, it is desirable to be familiar with the appearance and distribution of the mycelium. The sporophytic hyphae of the Gymnoconia are con-

[1] Accepted for publication May 2, 1923.
[2] Reference is made by number (italic) to "Literature cited," p. 242.

fined to the leaves in localized areas. The gametophytic hyphae become established in the perennial plant structures, from which they invade the new canes throughout their length and finally take part in the formation of aecidiospores. Newcombe (*8*) and Clinton (*3*) have given practically the only accounts which deal with the mycelium and haustoria of the orange-rusts in the host tissues. Both authors used the infected blackberry as the subject of investigation. Newcombe did not study sections of the roots, confining his attention to 16 cm. of an infected blackberry cane. He discovered that the bulk of the mycelium was to be found in the pith of the stem, noting hyphae in a medullary ray at one place. Clinton found mycelium in the fundamental tissue of the growing point, and although in old stems the hyphae were confined to the pith, they sometimes occur in the cortex and in the phloem of the bundles in young and growing shoots. He states that—

mycelial threads are present from the upper parts of the roots running through the stem into the uppermost leaves showing signs of infection * * *. Frequently plants are found in which the new shoots are affected but the old ones are free. In

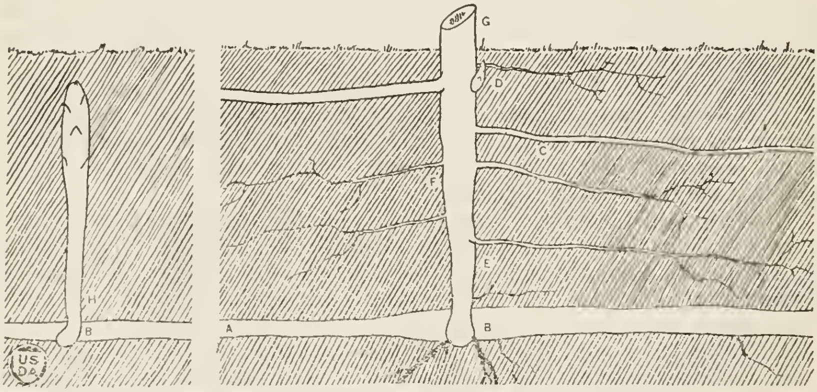

FIG. 1.—At the left, growth of shoot H from root runner. A, in April. At the right, same plant in October; B, root crown; small central pith would be found at E; horizontal roots, C, which happened to develop in this case do not ordinarily appear the first year.

such case the mycelium is found in the former only * * *. Sections of roots, except in the neighborhood of the merging of root and stem, do not show the mycelium.

Intercellular hyphae without septa or nuclei and the simpler types of haustoria are figured.

MYCELIUM IN THE BLACKBERRY

Clinton's account of the distribution of the mycelium is correct in general if applied to blackberries which have been infected for at least two years, except when he states that the roots of an infected plant do not show any mycelium other than in the transition zone where root and stem join. The writer has found that hyphae invade the roots of the blackberry very extensively, a fact which accounts for the rapid spread of the parasite to new shoots which rise from the roots. The blackberry forms a few large roots, some of them becoming runners which by budding give rise to new plants at some distance from the parent. These runners are true roots morphologically, their structure being the same as that of the ordinary root of the blackberry. If one carefully uproots rusted

blackberries growing together in nature, he often finds several infected plants attached to the same root. The writer has made sections of such connecting root runners and has found an abundance of mycelium in the medullary rays, in the phloem near the cambium, and in the cortex for many feet, or as far as the runner extends. Ordinarily but little mycelium will be found in the woody portion of the roots except along the medullary rays. More rarely hyphae are scattered irregularly in the wood; this usually occurs in the case of recent primary infection of root shoots by spores where there has been an increase in the amount of porous wood tissue formed as the result of the late invasion of the root by the parasite.

The distribution of the mycelium was traced in the canes and in the root system of a certain colony of infected wild blackberries at the time of year when rust pustules were maturing. There were found at a point (A in fig. 2) the remains of dead canes in the form of a witch's broom characteristic of plants infected by this rust. Large roots extended downward into the soil. From one root which was of the runner type

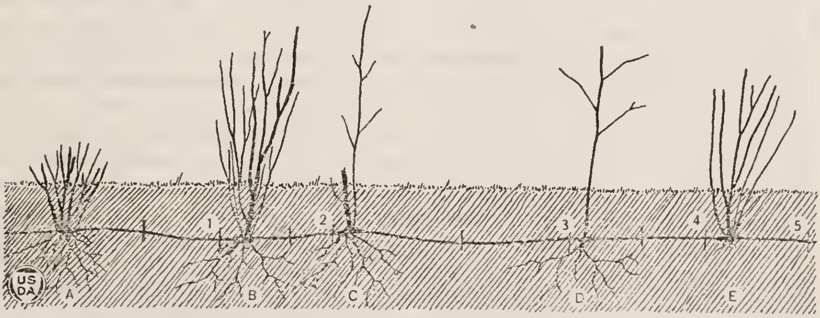

Fig. 2.—Diagram of a series of wild blackberry plants April, 1921, arising from the same root runner (part shown here about 6 feet long). A, dead canes of 1919 plant in form of a witch's broom; B, old cane (heavy line) and new shoots showing rust; C, stub of dead cane, new shoot rusted; D, old cane in blossom, not rusted; E, witch's broom consisting entirely of new shoots; sections of the regions 1, 2, 3, 4, and 5 of the root showed hyphae in the phloem and cortex. The plant D escaped infection through some accidental failure of the root mycelium to penetrate the shoot bud in time.

new shoots and old canes (B), now rusted, were arising at a distance of 18 inches. At C, 10 inches beyond, another plant, composed of rusted shoots, was attached to the same root; the old cane had died. Two feet farther (D) the plant from the runner consisted of one old cane in blossom and free from rust. At E, 14 inches distant, was a witch's broom of infected new shoots. Sections were made of the root runner at points on both sides of each plant; mycelium was found in the phloem and cortex in every case, demonstrating clearly the method by which the rust spread through the root system. Hyphae have been found in the ordinary type of root 18 inches below the ground. A plant arising from a runner bearing mycelium may rarely be devoid of rust (plant D noted above) due to the failure of the mycelium to enter the shoot at the time of its origin, just as rust-free canes are found in an infected hill. If the rust enters a shoot bud from underground parts, hyphae will later be found only in the pith of the cane, except at the nodes. In these regions hyphae may grow along the rays or outside of the wood ring.

The blackberries studied in the vicinity of Washington, D. C., and at Cameron, N. C., were infected with the short-cycled rust. Specimens of

Rubus canadensis, mountain blackberry, from Bartlett, N. H., infected with the long-cycled Gymnoconia show that the gametophytic mycelium of this rust spreads through the root runners in the same manner as that of the short-cycled form.

MYCELIUM IN THE DEWBERRY

The rust spreads in the wild dewberries in a totally different way. The trailing vines of the dewberry take root at the nodes or at the tips, and the mycelium in an infected plant, though confined to the pith in the differentiated region of the vine, follows the growing point and enters the buds as they are formed, so that new plants originating at these rooting nodes are infected from the beginning. Hyphae are present in that part of the stem which is buried in the soil, and, contrary to what might be expected, there is considerable extension of the mycelium in the root system. Sections of a dewberry infected with the short-cycled rust showed mycelium in the roots for a length of at least 8 to 10 inches.

The course of the mycelium was also followed in the root system of a wild dewberry infected with the long-cycled rust. No mycelium was found in the innermost wood ring. Hyphae were abundant in the tissues of the other rings, especially along the rays. The haustoria are often provided with short, more or less twisted and intertwined branches; in this case they are composed of several cells, each cell with a single nucleus. Such complex haustoria are more in the nature of intracellular hyphae, and nearly fill the cell attacked. The hyphae branch out in all directions on reaching the cambium and sieve tubes. Longitudinal sections show that the parasite advances toward the tip of the root along the cambium and phloem, certain hyphae growing out radially as the root increases in size. The presence of mycelium throughout the woody tissues of these roots does not mean that the fungus can invade the wood, once the xylem is laid down. Hyphae originally present along the inner side of the cambium layer are simply cut off, or surrounded, by new wood cells. The mycelium now forms an intricate network by which hyphae in the phloem and the living medullary rays are in direct connection with that part of the mycelium in the wood, so that it is not surprising to find hyphae fully alive embedded in the wood of old roots. Passing from the primary to the secondary and tertiary roots, the mycelium is more and more confined to the outer part of the wood cylinder and phloem, until in the smaller rootlets 1 year old the writer found hyphae only in the soft tissue outside of the wood cylinder—that is, along the cambium, phloem, and in the cortical parenchyma—indicating that practically all forward growth of the fungus takes place in the latter tissues. Certain roots of an infected plant may escape invasion by mycelium, and rust-free vines in such hills are not rare.

MYCELIUM IN BLACK RASPBERRY

Theoretically, the habit of the black raspberry cane which enables it to develop into a stolon which roots at the tip ought to serve admirably for the distribution of the long-cycled rust to new plants. In order to learn whether this were true, several rusted wild black raspberries have been marked and observed for three years. Not infrequently the rusted plants are so severely attacked as to die the second or third year. Again, the canes in the infected plants are of the witch's broom type and they

are not apt to become stolons and take root. In such plants the disease "runs out" because its host dies. Occasionally, some of the infected canes recover vigor and become stolons, in which case the mycelium passes from the stolon into the buds which arise to make new shoots at the point of rooting, thus spreading the disease to new plants. Sections of the internodes of infected canes and of such stolons have always shown mycelium in the pith, none in the phloem or cambium. Further consideration will be given this point in another connection. The distribution of the hyphae in the underground parts of the infected black raspberry is practically the same as that found in the blackberry and dewberry, except that hyphae are very much more abundant in the woody tissues of both roots and stems of the raspberry. One black raspberry examined had been under observation for three years. Sections of the large vertical underground stem bases from which the spindling canes were now arising, showed mycelium in the pith, scattered irregularly through the wood and along the medullary rays in the xylem and phloem regions. No hyphae were found in the well-marked pith of the much enlarged and distorted rhizomelike structure from which roots were growing.

The largest roots were only about 1 foot in length. The fungus was found throughout the wood, and especially along the rays for a distance of 8 inches in one root; and the presence of hyphae was demonstrated in seven other roots, each cut at about 3 inches from its point of origin. The fact that the dewberry and black raspberry are not propagated by root sprouts precludes the possibility of the spread of the disease through the roots. It is clear that the invasion of the roots by the mycelium is a matter of nutrition and is not correlated with the spread of the disease, as one might be led to believe if only the blackberry were studied.

Although the orange-rust has been reported on the red raspberry, *Rubus strigosus*, the writer has been unable to obtain specimens for an investigation of the distribution of the mycelium of the systemic stage in this host.

LOCAL INFECTION OF RUBUS WITH AECIDIOSPORES OF THE LONG-CYCLED RUST, GYMNOCONIA INTERSTITIALIS

In order to obtain a supply of teleutospores for experimental purposes, it was found desirable, for example, to infect the black raspberry by sowing aecidiospores of the Gymnoconia from this host. It is well known that young apple leaves are much more liable to infection by the cedar-rust than are the older or more mature leaves. It was with this in mind, as well as to obtain shorter plants for inoculation, that the writer at first purposely pruned away the old canes in the potted plants used in the greenhouse work. Plants in nature having young shoots on which leaves were just unfolding were chosen in preference to those with the old canes present or with leaves that were more fully grown. Judging from the results which followed, both practices were unwise, since it was found that the leaves on the old canes and the lower leaves on the new canes frequently bore telia, while the leaves which were just unfolding at the time the spores were sowed rarely became infected. The experiments were repeated the two following years, and in each test the tip ends of the raspberry canes were tagged at the points where the leaves were just beginning to unfold. Below the tags the leaves were fully expanded. Furthermore, the old canes were not cut away,

so several types of leaves with respect to age and position on the canes were exposed to infection. The leaves on the old canes were the most susceptible each year.

Leaves of the black raspberry were infected with the telial stage by sowing aecidiospores from the blackberry, and the southern dewberry, *Rubus enslenii*, was likewise infected by sowing aecidiospores from the black raspberry. Other species or varieties of Rubus said to be immune, or at least very resistant, to the orange-rust stage were infected with the telial stage.

The conditions under which leaves of Rubus are most readily infected with the sporophytic or "*Puccinia peckiana*" stage is outside the scope of this paper. This phase of the work is being considered in another paper on the effect of orange-rust on the development and distribution of stomata.

SYSTEMIC INFECTION OF RUBUS OCCIDENTALIS WITH SPORIDIA OF THE LONG-CYCLED RUST

In infection experiments with the systemic stage of these rusts, the ordinary means of checking results by the use of isolated control plants alone does not provide sufficient safeguard. No one can explain or account for the results reported by Atkinson (*2*) on any other basis. A blackberry or raspberry may be infected in its underground parts without showing the rust on the aerial parts every year, so it would be unsatisfactory to use as experimental plants those which have just been received from a nursery or have been dug up in nature. An infected plant may be transplanted during the spring before the leaves are out and develop leaves normally in the greenhouse without showing rust that season, especially if it recovers only slowly from the shock of transplanting. Since the presence or absence of mycelium in a cane or root can be demonstrated without question, one can be certain by using only tip shoots obtained from rooting canes that the raspberry which he inoculates is uninfected at the beginning of the experiment. It is not necessary to provide control plants in checking up the results.

METHODS

The methods used in preparing sections of canes or roots to demonstrate the presence of mycelium are the same as those adopted in the writer's work on Gymnosporangium. Transverse sections 10 to 50μ thick stained with acid fuchsin and iodin green show hyphae and haustoria very plainly under the microscope. Longitudinal sections show hyphae much better, especially where they burrow along between the cambium and the phloem—for example, near the nodes of blackberries which have been primarily infected. It is very easy to overlook the hyphae in the cambium region if one does not prepare longitudinal sections.

The habit of the black raspberry mentioned above, whereby the canes take root at their tips, provides a very efficient natural method of propagation. Since it is possible to examine these stolons to learn whether or not the fungus has in some way established itself previously in the plant to be used, it has been found advisable for purposes of inoculation to use the tip plants.

The tip ends of some of the raspberry canes were wired to the soil in ordinary flower pots as soon as they began to mature sufficiently to take root. After a few days, leaves of black raspberry bearing teleutospores were laid over the rooting tip ends and kept moist under muslin damp chambers for several days.

INFECTIONS IN THE GREENHOUSE

About the middle of January the inoculated plants were transferred from the cold frames to the greenhouse. As soon as new shoots appeared it was evident that a number of the experiments had been successful. Fifteen plants which had been propagated by rooting the tips of rust-free plants were inoculated during August and September, 1921. Nine of these plants developed aecidia the following spring. An examination was made of the cane from which each new plant had been derived, material for sections having been obtained at the time the new plant was separated from its parent. No hyphae were discovered in any of these canes. As a double check when the rust began to show, another set of sections was made of that part of the original stolon still attached to the new plant. Sections were also made of the tip ends of such canes as had grown forward after they had taken root, so it is evident that the nine plants now showing rust were uninfected at the beginning of the experiment. The parasite must have gained entrance in each case as the result of inoculation with

Fig. 3.—Diagram of rooting tip of black raspberry, *Rubus occidentalis*, No. 215, which was infected by laying leaves bearing telia over the tip. Shoots G and F bore pycnia at this time. Shoot buds C, D, E, and I were infected, but still beneath the soil; L, dormant bud. Sections at A and B showed no traces of mycelium. Sections of the stolon at L, O, K, and K′ showed hyphae along the cambium and phloem, none in the pith. The young shoot buds, C and I, had hyphae in pith as well as in the growing region and among the bundles where differentiation was incomplete.

sporidia from teleutospores and not from hyphae carried over from an infected parent plant. It was pointed out previously (p. 213) that in the internodes of an infected cane the mycelium is confined to the central pith. At or near a node, hyphae are sometimes found growing along the medullary rays in the wood and in the cambium and phloem regions. In any case, there will always be some mycelium in the central pith. It is due to this characteristic distribution of the hyphae that it is possible to distinguish between cases of primary infections by sporidia and secondary infections where the mycelium enters a new cane directly from the infected parent plant.

On February 18 one of the artificially infected plants, No. 215, was freed from soil and photographed (Pl. I), and a diagram (fig. 3) was made in order to locate the regions or structures from which material was obtained for sections. The new shoot buds, figure 3, C, D, and E, 3 mm. to 1½ cm. long, were pushing out, but had not reached the surface of the soil and were still without chlorophyll. At L there was a dormant axial bud near the base of which a few roots were attached. Sections of the stolon at B and of the roots and dormant bud at L showed

no hyphae, but several hyphae having haustoria could be seen running between the cambium and phloem of the cane near the point of origin of this bud. It is clear that if the bud had developed it would not have been infected because of the very effective barrier of indurated tissue which was present between the hyphae and the bud. The next year an uninfected cane would have been seen in a hill where the other canes were rusted. The location of the mycelium in the stolon was also determined by sections at O, K, and K′. In each place the hyphae were confined to the region of the cambium and phloem, being absent in the pith wood and cortex. Sections of the basal portions of the infected shoots F, I, and C showed that the mycelium is much more generally distributed in the pith, phloem, and cortex of these young structures. The absence of mycelium from all tissues at A and B and in the dormant bud L, taken in connection with the general distribution of hyphae in the shoots, indicates that this plant was first infected in an axial bud which later gave rise to a shoot such as F. The mycelium after entering the tissue at the base of the bud made its way along the cambium and phloem in both directions, entering new bud primordia as they developed in the region where the tip was taking root. The growing tip of the stolon was not infected, for no hyphae were found in cross sections at A. It is clear for the following reasons that the experimental plants were rust-free when inoculated: (1) The parent plants have been observed in the greenhouse three years, and still show no rust. (2) No mycelium was present in sections of the stolons giving rise to the experimental plants, these sections being made from pieces killed at the time the new plants were cut away from the parent. (3) A second series of sections was made from the piece of stolon attached to the new plant after the rusted leaves had appeared the following spring. These parts contain no mycelium. (4) Sections of the tip ends of such stolons as continued growth after rooting showed no mycelium.

Further investigation proves that it is essential to section the stolon only at the place where it has taken root or where the infected shoots originate, in order to determine whether a rusted tip-plant has been primarily infected by sporidia, or has been invaded by hyphae from its infected parent. If there are no hyphae in the central pith of the parent cane where it roots one can rest assured that it is a case of infection by sporidia. The mycelium will then be found along the cambium and phloem.

During the time in which the tip of the stolon is establishing a root system, buds are also developing. Since the fundamental parenchyma of the bud primordium at this time is not well protected with scales and the wood ring is still unformed, hyphae from spores falling on these parts might easily find their way into the growing regions and penetrate to practically all parts of these buds, and from here grow back along the cambium region. The cambium and phloem tissues are especially well provided with food which enables the fungus to establish itself outside of the xylem ring in the phloem or cortex, from which it readily makes its way into the new buds as they are formed in this region.

The plants that escaped infection in the writer's inoculation experiments appear to be the ones in which the stolon after taking root grew on to some extent without forming shoot buds, so no means was afforded at that time for the entrance of the fungus into the host. The growing tip of a stolon is certainly a very tender structure which one would think might be attacked by the parasite. If this happened in any of the ex-

periments the tip must have stopped growing forward or died, since in every case in which the tip grew beyond the rooted plantlet, it contained no mycelium. Certain stolons rooted early so that the shoots were of considerable size before teleutospores were available and were undoubtedly too old to become systemically infected.

The experiments, described above by which tip plants were systemically infected were repeated during the summer of 1922. Teleutospores for this work were obtained from blackberry leaves. This attempt to infect the raspberry with the strain from blackberry proved successful; four of the tip plants showed orange-rust the next spring.

FIELD EXPERIMENTS

Leaves bearing teleutospores were laid over rooting tips of a number of wild raspberries in the field. The rainfall in this region was so limited in August and September, 1921, that these rooting tips rarely formed buds or shoots until the following spring. Only one plant was infected in this way.

PRACTICAL CONSIDERATIONS

PROPAGATION OF NEW PLANTS FROM DISEASED STOCK

It is possible for an infected raspberry cane to become a stolon which by rooting will spread the disease to new plants in the nursery. The characteristic appearance in nature of plants heavily rusted suggests that this is not a common occurrence because so many infected canes are of the broom type, short and spindling; and it is well known that the entire plant frequently dies from the effect of the rust. On the other hand, as noted previously, rusted canes may recover their vigor and many shoots which arise late in the season do not, of course, show any outbreak of the rust. Several rusted raspberries in nature and in the greenhouse were observed in 1921 and later. In many of these no stolons were formed, but occasionally canes clearly diseased did take root at the tips and gave rise to new plants. In several such cases the rooted plants were cut away from the parents and pieces of the stolons were sectioned. It was found in each case that the mycelium was present in the pith of the cane which had rooted at the tips, and that it must have entered the buds from which the new plants developed because they showed the rust the following spring.

RUST-FREE PLANTS FROM INFECTED STOCK

One commonly observes that some young canes in an infected hill do not show rust. When no mycelium can be found in such canes, it is clear that they arise from a part of the crown into which the fungus has not penetrated. These canes taking root at the tips must give rise to perfectly healthy plants. An infected plant was brought from Massachusetts, in June, 1920. A stolon was formed after the plant had apparently recovered from the rust. This stolon was examined after it had taken root at the tip, but no mycelium was found. The new tip plant showed no rust in 1921. It would be bad practice in a nursery to use such plants for stock, because, as noted above, mycelium may exist in a cane which shows no signs of the rust at the time. New plants arising from rooting stolons may be readily infected in August and September, but if the tip

plants have grown to considerable size before inoculation is possible, infection is unlikely to occur, This being known, methods can be easily worked out for the insuring of propagation of rust-free nursery stock.

TIP PLANTS INFECTED IN NATURE

If it is possible to infect "tip plants" of *Rubus occidentalis* experimentally with the systemic stage of the rust, what is the evidence that this is the method by which the disease is spread in nature? If one examines in early spring wild black raspberries in a region where infected plants are known to have existed in the past, he will find rusted tip plants arising from stolons which are connected with rust-free parents. Sections of such old stolons will show no hyphae. This originally suggested to the writer the probable method of primary infection.

OLD PLANTS SUBJECT TO INFECTION

There remains a question as to whether the rust is able to gain entrance into an old plant. The writer's experiments along this line have not been as satisfactorily checked as is desirable. Eight plants were obtained from a region where rust was known to be present. They showed no rust in 1921 after they were planted in the greenhouse. Since they were very small and had new shoots starting out from the old crown at the time when the first teleutospores were available, they were placed under favorable conditions for infection. Having been overwintered in the cold frame they were again brought to the greenhouse, where shoots bearing pycnia made their appearance on three of the plants within a few days. Apparently the inoculations had been fairly successful, but one can not be certain that the plants were not infected at the time they were transplanted.

In nature the new canes spring up from the base of old plants early in the season, so the gametophytic stage of the long-cycled rust naturally attacks the tip plants, which are in the most susceptible condition from the latter part of July to September, and not the basal shoots from old canes which arise in the spring. It should not be forgotten that teleutospores sometimes mature on leaves of the old canes a very short time after aecidiospores are shed, no doubt early enough to lead to infection of the more tardily formed basal shoots. It is not an uncommon practice among growers to prune out all the old canes and some of the new ones after the crop is harvested. New shoots might, as a result, grow up from the base of the old plant following this treatment, so that the conditions would be favorable for infection by sporidia from telia which would be mature at this time. There is still another possibility. The teleutospores of Gymnoconia are known to be capable of germination as soon as mature. The writer's experiments prove conclusively that some of these spores germinate in August and September if placed under suitable conditions. On the other hand no one has proved that the teleutospores may not also live over winter and be in condition for germination as the first new buds or shoots break through in early spring.

It has been pointed out that rusted canes do not ordinarily become stolons and set tip plants, so the rust is not spread in this way to any great extent. New shoots arise from the base of the old plants in the spring, while the teleutospores mature and germinate from July to September, the same time that the canes root at the tips. It must be concluded, therefore, that in nature the perennial stage of the long-cycled

orange-rust on black raspberry ordinarily spreads from plant to plant by means of sporidia which infect the rooting tips of canes in August and September.

SYSTEMIC INFECTION OF THE BLACKBERRY WITH SPORIDIA OF THE SHORT-CYCLED FORM

There are clearly two strains of orange-rust on blackberries, the short-cycled form, "Kunkelia," and the long-cycled form, Gymnoconia, which has *Puccinia peckiana* as its teleutosporic stage. The writer is describing elsewhere blackberries that are infected with orange-rust of such a nature that spores in one aecidium all develop promycelia, and those in some adjacent aecidium all produce germ tubes. One should perhaps hesitate before adopting separate generic names even for the strains that appear to be well fixed. It certainly is to be regretted that the aecidiospores of the "Kunkelia" are sometimes referred to now as "teliospores." This practice not only leads to confusion but has no basis in morphology. The pycnia of the short-cycled rust are said by Arthur (*1*) to be subcuticular. They are, of course, sub-epidermal, the same as are the pycnia of the Gymnoconia. Newcombe (*8*) and Clinton (*3*) illustrate sub-epidermal spermogonia of the orange-rust.

METHODS

During April and the first weeks in May, blackberries at the Arlington Farm, Va., send up shoots in large numbers from roots. By digging about shoots that have appeared above the ground, one can find white or reddish shoots in still earlier stages of growth. Some of these young shoots may be broken off or injured as one endeavors to uncover the youngest, but he can readily find places where a half dozen or more ranging from mere buds to shoots 6 or 8 inches high, are growing in a space covered by the infection frame. A large pan is set on sticks or on a frame at the desired place, and muslin strips are hung over the frame dipping into the pan, which is filled with water. This is the iceless refrigerator designed by Hunt (*6*). If the apparatus is shaded from the sun and the pan kept filled with water, a very efficient damp chamber will be provided for field work. Several methods of "inoculation" were tried, aecidiospores in each case being obtained from wild blackberries growing at Radnor Heights, near Rosslyn, Va.

AECIDIOSPORES SOWED ON SHOOTS

Leaves bearing aecidia were laid over young shoots, so that spores were shed naturally over growing tips, etc., or the spores were dusted over the shoots and into the leaf axils. In this way an excess of moisture which prevents proper germination is avoided. Aecidiospores were also sprayed on the shoots or injected into growing tips, leaf axils, etc.

SPORIDIA ATOMIZED ON SHOOTS

Aecidiospores were germinated on water in watch glasses, or on agar, and the sporidia thus obtained in large numbers were sprayed on the young shoots. It is clear that the use of sporidia instead of aecidiospores will prove to be more satisfactory in infection experiments since the time during which the plants must be kept in the damp chamber is shortened by at least 24 hours. In the writer's experiments, plants are

kept under the infection frame two days when sporidia are used, and three or four days when aecidiospores are sowed.

INOCULATION BY MEANS OF THE HYPODERMIC NEEDLE

A number of attempts were made to infect young shoots by injecting spores into their growing tips with a hypodermic needle. In several cases infection followed in spite of, rather than because of, this method. The needle was loaded with an abundance of sporidia and generous doses were given the young shoots. Water containing sporidia must have trickled from the wounds over the surface of the young shoots, which were exposed by digging away the surrounding soil. The injury to the growing tips manifested itself the following year. The ends which had been punctured usually died and new shoots developed from below.

INOCULATION IN THE OPEN

During rainy weather when the plants remained moist for the greater part of a day or two, inoculations were made with great success by spraying young shoots with sporidia without the use of artificial methods for maintaining the humidity. These experiments were conducted somewhat apart from those which were more nearly under control through the use of muslin infection frames.

CONTROLS

For reasons which will be made clear later, definite control plants were not set aside in this work, although all plants of the same horticultural variety in near-by hills could be considered as control plants, especially where no damp chambers were used. Plants in well-cultivated fields do not serve well as controls since so many of the young shoots are intentionally destroyed in cultivating, and these young plants would be the ones most readily infected by spores from wild blackberries in the vicinity. The destruction of the young shoots that grew up between the hills and rows in the field was avoided in order that use might be made of as many of them as possible as an additional check on the results. Most of the infection experiments with blackberries for 1921 were made in the field plots where exposure to natural infection must be taken into consideration, and it should be remembered that greenhouse conditions in this regard are only slightly more dependable in a region where the orange-rust is so common that vast numbers of spores are very likely to be blown into the ventilators of a greenhouse. One can not be quite certain, therefore, in this vicinity that any particular plant may not have been infected by these wind-borne spores.

It has been shown previously how the rust may be carried from plant to plant through the root runners, so in a group of wild blackberries in which a large number of the plants are rusted it may be that the number of infected plants had increased year by year, as roots containing mycelium gave rise to new shoots. A single primary infection might easily account in this way for conditions where nearly every plant in a stand of wild berries is rusted. Therefore, as an additional safeguard after the writer's plants had been received from nurseries during April and May, 1920, and also in 1921 when the first inoculation work was done, a careful inspection was made from day to day. The work was, therefore, begun with plants whose canes showed no rust the first and second seasons of their growth. For example, a stock plant set out in 1920

develops a horizontal root 1 or 2 feet long, from which arises, in 1921, a shoot in condition for infection. If inoculation is successful, it will be manifested about April, 1922, by the appearance of rusted shoots from the base of the 1921 plant. If examination of sections of the horizontal root connecting the parent plant with the one inoculated fails to show hyphae, it is certain the rust did not come from the parent.

The mere fact that a plant shows no rust on its leaves is not proof that the mycelium is not well established in its crown or in the root runners; but if the runner bearing an infected cane shows no mycelium, the infection must be a new and independent one, regardless of the fact that the parent plant may also be rusted. In this way it will be shown that a root runner may bear two or three infected plants along its course and each plant be separately and independently infected.

Inasmuch as potted plants can not send up shoots from roots at an appreciable distance from the parent plant, they are not as satisfactory for infection experiments as are those grown in "flats" on the bench or as are field plants where shoots are formed on roots 2 or 3 feet from the parent. The Iceberg variety appears especially favorable for the production of root shoots. By digging in the soil, still younger shoots can be exposed when they are in a very susceptible condition.

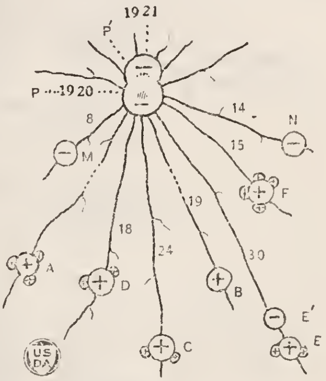

Fig. 4.—Diagram showing arrangement of inoculated plants and roots connecting them with parent Crystal White blackberry, No. 134. Nursery stock, P, P', planted in 1920. Other plants indicated, larger circles, were young shoots in May, 1921, when the experiment was made; small circles attached, new shoots, 1922. Infection frame probably covered area about NEA. Numerals represent distances in inches. Signplus + indicates plant infected May, 1922, when dug; roots bearing plants A and B broken off in digging; sign − indicates uninfected plant. See text and figure 5 for further explanation.

As an illustration of this phase of the work attention is directed to figures 4 and 5 which are diagrams of a part of the root system of plant No. 134, May 6, 1922. The inoculation frame would have covered the six plants, figure 4 (+), which became infected and probably took in either M or N (−) which were not infected. Plant P' was not inoculated although it was a young shoot 5 or 6 inches high when the others were inoculated May 23, 1921. Figure 5 shows how clear-cut the original parent nursery stock remained. There are no shoot buds present on it such as are always found on this vertical underground structure when it harbors mycelium of the orange-rust. The roots N and C arise close together, yet C now bears an infected plant while the one from root N is rust free. The convincing evidence that the parent plant, P, P', is not already parasitized is obtained only by making sections of the root bearing the new plant.

RESULTS

When one studies blackberries in nature, he becomes familiar with those which are infected, and can recognize them after the leaves have fallen. Even though the canes may not be particularly stunted, their appearance and the size of the buds are such as frequently to enable one to pick out the diseased plants. The writer's experimental plants were therefore examined with considerable interest at intervals from June until November following the inoculation experiments. Only one plant was found, September 28, which suggested that the inoculation had been

successful, whereas in reality over 120 separate infections had occurred. The failure to pick out the infected plants lay in the fact that it is not until the second year the rust has lived in the blackberry that the canes become so infected secondarily by invasion of mycelium from the peren-

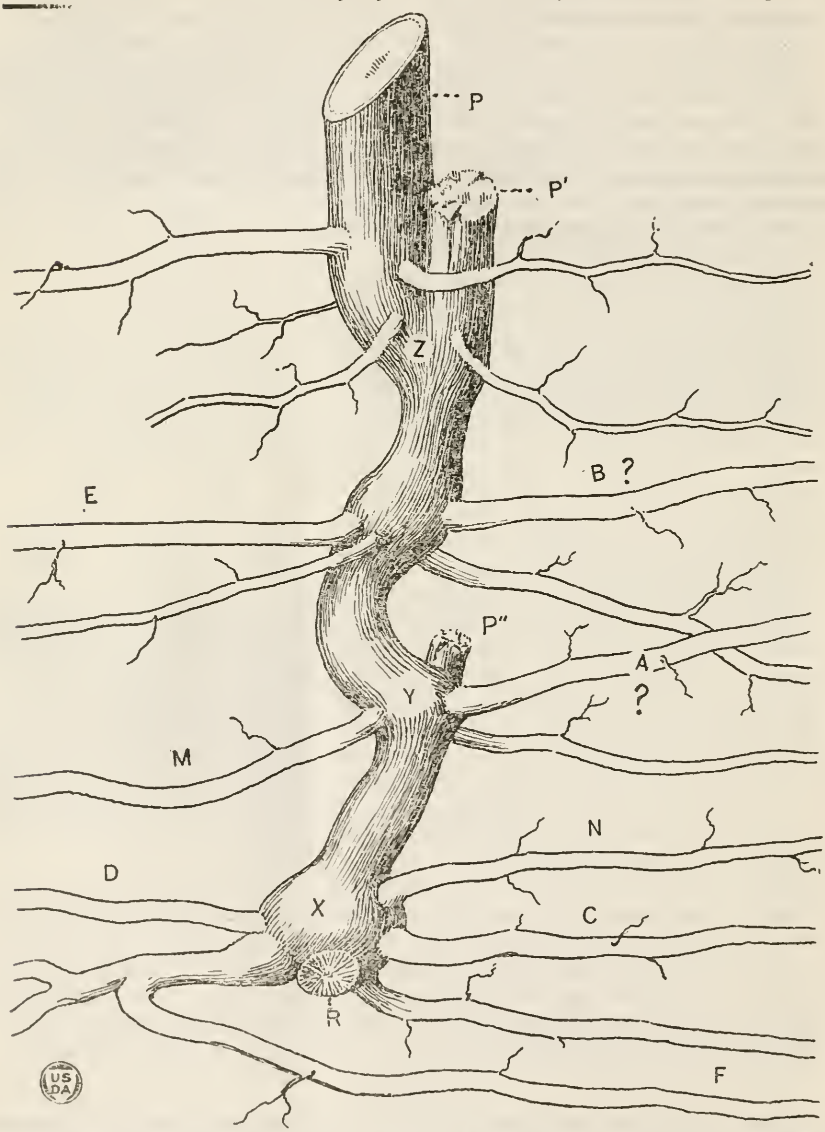

FIG. 5.—Root system of nursery stock of blackberry No. 134 planted May, 1920, dug May, 1922. Lettering has same significance as that in Figure 4. Infected plants A, B, C, D, E, and F arise from roots connected with the original stock at three different levels. The roots shown here are entirely free from mycelium. See figure 4. Original root crown at X, present crown at Z.

nial base that they show the peculiar features mentioned. Canes primarily infected remain for the most part quite normal and blossom as usual, since the upper parts are free from the parasite. The results of the infection experiments are given in Table I.

TABLE I.—*Primary infection of the blackberry with sporidia of the short-cycled orange-rust, 1921*

Plant No.	Variety.	Time inoculated (1921).	Infection noted (1922).	Number infected.
92	Eldorado	April 30	April 28	1
93	....do	....do		0
94	Crystal white	April 23	April 29	2
95 C. W.	....do	April 16	May 9	2
97	....do	April 30	April 30	4
112	....do	May 5	April 28	3
134	....do	May 23	May 15	5
336	....do	May 2	May 6	2
337	....do	April 27	....do	3
342	....do	....do	May 3	5
95	Kittatinny	April 16	April 28	1
98	....do	May 2	April 14	9
135	....do	May 23	April 28	1
344	....do	May 15	April 30	5
353	....do	April 20	May 5	2
354	....do	April 28	....do	2
355	....do	April 24	....do	2
356	....do	April 25	May 16	3
357	....do	....do	May 5	5
96	Lawton	April 30		0
99	....do	May 2		0
101	....do	April 23		0
105 B	....do	....do		0
125 B	....do	....do		0
136	....do	May 23		0
154	....do	June 8		0
330	Mercereau	May 2	April 29 May 17	2 5
331	....do	April 24	May 20	4
343	....do	....do	April 30 May 8	2 7
352	....do	April 25	May 8	3
369	....do	....do	May 9	1
370	....do	....do	....do	2
371	....do	April 30	May 19	2
372	....do	....do	May 9	5
373	....do	April 26	....do	4
377	....do	....do	May 11	1
333	Iceberg	April 30	May 5	1
334	....do	April 20	April 28	5
335	....do	April 30	....do	1
338	....do	....do	May 2	2
340	....do	May 2	May 1	2
341	....do	April 23	May 11	3
345	....do	April 27	May 1	1
358	....do	April 23	May 3	1
359	....do	April 21	May 2	3
360	....do	April 26	May 17	3
393	....do	May 2	May 25	1
361	Crandall	April 23	May 3	6
415	....do	May 2	May 22	1
422	....do	....do	May 25	1

The results of inoculation work carried on in 1922, repeating many of the experiments reported in the table, furnish further evidence in support of the writer's interpretations and conclusions as regards the various types of infection.

TYPES OF INFECTION

The value of infection work lies not so much in the fact that one acquires the technic of infecting the host, as in the knowledge of the host-parasite relationship to be gained by a study of the plant structures under most favorable conditions. During April and the first week in May, 1922, as an infected plant became noticeable, it was dug up so as to include the root from which it had sprung, and also, in many cases, all other plants connected with the same runner. Pieces of the canes and roots at various points were fixed in Flemming's Medium Fluid and later sectioned and stained. After a careful study of many phases of the question, it was seen that infected plants could be arranged in three or four groups, based upon the manner of the primary attack by the fungus and the reaction of the plant to the attack.

A. This group includes those plants which were, as shoots, infected in or near one or more axial buds below the growing region, so that the shoot grew into a normal cane which blossomed the next spring and would have borne fruit if allowed to live. Rust appeared on the leaves (of the old cane) at a few of the lower nodes as the result of a more or less local infection. New shoots, systemically infected, would arise from the base of the old cane, the one originally inoculated, provided the hyphae had penetrated downward into the underground part of the stem or root. There should be, therefore, in this group three subclasses based on the extent downward to which the hyphae had penetrated: (1) Those infections which were so localized as to be unable to reach the underground parts; the result would be a local infection which would disappear with the natural death of the cane at the end of the season. (2) Those cases where hyphae penetrated through the cambium or phloem down the stem beneath the soil sufficiently far to stimulate the development of new shoots into which the mycelium could penetrate and live over another year. By merely pulling up such canes so that they break off at the root would be all that is necessary to free the plant from the rust. If such a plant is allowed to live through the spring, however, the rust would be able to establish itself permanently in the crown and roots. (3) The third type would include all cases where the root has been reached by the fungus the first season. In such types the connecting horizontal root must be destroyed.

B. The small number falling in the second group includes those plants in which the fungus soon became established in the growing region of the shoot or axial bud, with the result that the whole cane or branch became systemically infected. Such types can easily be recognized the following spring by the appearance of the new shoot which arises at the end of the old cane, or its branch, by proliferation of the terminal bud which has remained alive over winter.

C. The third group includes those types in which the root was directly infected so that the fungus entered the phloem. In such cases the root lay near the surface of the ground. Such a type of infection results in the formation of a witch's broom at once and the rust becomes fairly well established. Since the mycelium in such cases has probably not penetrated either way in the root more than a few inches, the parasite can be destroyed by pulling out the section of the root which bears the infected shoots. Each of these types of infection will now be considered in detail.

PRIMARY INFECTION BY THE SPORIDIA NOT BECOMING SYSTEMIC

Local infection with the sporophytic stage by aecidiospores is, of course, the rule with the Gymnoconia. The question has frequently arisen whether local, as contrasted with systemic gametophytic infection by sporidia, may not sometimes also occur. One frequently sees rusted leaves on certain branches or nodes, while the leaves at all the other nodes of the same cane remain free. The writer made sections of several such naturally infected canes at points above and below the nodes bearing rusted leaves. Having found hyphae in the pith in each case, he came at first to the erroneous conclusion that infection by Kunkelia is always systemic, the rust appearing more tardily on some leaves, or not at all if the mycelium happened not to penetrate the leaf primordium at an early stage. Later an exception was noted which remained a puzzle until the results of inoculation experiments became available. A sand blackberry at Cameron, N. C., was found May 2, 1921, with rusted leaves at only one or two nodes of its single "old" cane, which was otherwise perfectly normal. Sections of the cane at the base and at points above the affected nodes showed no hyphae in the pith, indicating that in nature "local" infections are possible. The proof of such infections has become conclusive as artificially infected plants have been studied. One clear case noticed was that on plant No. 24, a wild blackberry showing no signs of infection February 14, 1921, when it was transplanted. After it was potted it grew vigorously, showing no rust during the year. On March 22, 1921, it was sprayed with sporidia and kept under a damp chamber three days. The plant was taken from the cold frame January 12, 1922. Leaves soon appeared on the old canes and new shoots grew up, but the plant was perfectly normal, no rust having developed. It has been found that when infected plants are kept from year to year, the leaves of new shoots that push out a few days after the plants are brought into the greenhouse are usually covered with pycnia before they unfold. It was, therefore, surprising to find two months later (March 19) that rust was beginning to show on this plant. The tip of the old cane had died during the winter, but from the uppermost living node a new shoot had recently developed. The leaves of this new branch now bore aecidia. Sections at the base of this cane showed no mycelium in the pith, phloem, or cortex. Just below the infected node, however, hyphae were found in the cambium region and along the medullary rays, but none in the pith. A critical study of the distribution of the mycelium in the cane showed that the parasite had originally gained entrance through an axial bud which must have been at least 3 inches above the ground. The end of the cane had died during the winter, but hyphae were making their way slowly down the cane in the region of the cambium, which it was stimulating to renewed growth. This was indicated by a second annual ring of wood which was being formed (Pl. 2, D, y), the cane at this point being larger than at the base. The plant was then set out in soil on the bench in the greenhouse, where it has since grown vigorously without showing any signs of being infected. It is clear that the cutting out of the one cane which showed rusted leaves at a single node freed the plant entirely from the parasite. Had the infected cane been allowed to remain, it is doubtful whether the hyphae could have reached the perennial parts below the ground before the cane would have naturally died. Several other cases,

some of which will now be described, of similar types of local infection were met in connection with inoculations in the field which leave no doubt that "local" infections do take place when they occur at some point on a shoot several inches above the ground.

Two shoots, No. 353 A and B of the Kittatinny variety, about 4 inches high, were sprayed with sporidia May 5, 1921. These grew into large canes 5 feet tall and blossomed profusely in 1922, but no shoots, such as usually characterize infected plants, grew out from the lower parts of the canes during the spring. The fact that both plants had been slightly infected might easily have been overlooked. Each had been infected at a single node, and the fungus had failed to make any material advance upward or downward in the cane. The small branch of 353 A, was only partly infected; several blossoms appeared at the end. The parasite had attacked plant 353 B (Pl. 3) somewhat more vigorously because all of the leaves of two small branches from the infected node showed the rust. Sections of the canes above and below the infected node would show no hyphae. These attacks were very limited in extent and could not have succeeded in permanently infecting the plant. Plate 3, A, shows local infections in a Crystal White blackberry.

Some very striking cases of localized primary infections of the wild dewberry *Rubus enslenii* were noticed at Salem, N. C. Several plants showed rust only on leaves at one or two nodes; the rest of the vine in each case appeared to be perfectly normal. Longitudinal and transverse sections of the vines at about 2 inches above and below the infected nodes were made, but no trace of mycelium was found at these points. There can be no doubt that they were primary infections, and of a local nature, reminding one of the way the Calyptospora attacks the blueberry. The shoots from the infected nodes of these dewberries were very severely attacked. Rust pustules broke out along the young shoots, on the pedicels, and on the calyces as well as on the leaves. The conditions at the time they were infected must have been particularly suitable for the type which the writer calls localized gametophytic infections which fail to become perennial. The same spot was visited in May, 1923, and it was found that the rust had become established in only one of the plants. The shoots must have been rather well developed when the sporidia were being shed.

In 1922 sporidia were sowed on a number of Taylor blackberries whose new canes were from 3 inches to 2½ feet high. This variety proved to be very susceptible. The results indicate still more clearly that canes can be infected with the orange-rust even after they have reached a height of a foot or more. Eight of the canes that showed rust in the spring of 1923 were infected at nodes now 18 inches to 2½ feet above the soil (Pl. 5, B). In some canes the absence of hyphae in the internodes proved that separate infections had occurred at adjacent nodes. It will be shown later that had these plants been grown in flats in a greenhouse, the mycelium would have spread pretty generally through the canes and into the roots because of the conditions which contribute toward etiolation and thus prevent the canes from entering the dormant condition until later. A vine of the dewberry, *Rubus hispidus*, was found infected at only one node. No mycelium was seen in sections of the adjacent internodes. Cutting away the infected node freed the plant entirely. It has grown two years since without showing rust.

LOCALIZED SYSTEMIC INFECTION MAY BECOME PERENNIAL

Slight infections which may become constitutional if the canes are not destroyed are represented by No. 334 F (Pl. 4, A). The shoot from which this plant had developed was originally infected near the soil level after inoculation April 23, 1921. A normal cane developed during the summer. Two or three new shoots, systemically infected, grew from the base of the cane the following spring, and rusted leaves were also noted at the lowest nodes of the old cane May 3, 1922. There were no hyphae in the root, 12 inches nearer the parent plant, which was set out in 1920. This figure illustrates a common habit of growth of such horizontal roots which become larger after having given rise to a new cane. Fully two-thirds of the successfully inoculated plants of the Iceberg and Mercereau varieties had much the same type of infection as the one just described. If one merely pulls the cane from its horizontal root the spring following infection, all of the structures containing the parasite might be destroyed, but if in this case the plant had been allowed to live, the parasite would have invaded new roots and shoots and become thoroughly established. Should the fungus have found conditions more favorable during the summer for the extension of its mycelium downward, the type of infection illustrated by No. 112 C, described below, would have been found. Plate 4, B, illustrates a somewhat vigorous infection of the same type. The tip end of the shoot originally inoculated soon died, but this did not interfere with the activities of the parasite. An axial bud below immediately developed and is now represented by the large cane which is about to blossom, only the basal nodes of this old cane being infected. Three systemically infected shoots arise from the part beneath the soil. If the primarily infected old cane is broken off or cut away during the winter or in the spring before the sap begins to flow, a larger number of new shoots such as are shown in Pl. 5, A, will develop, making a witch's broom type of infection.

PRIMARY INFECTION BECOMES ESTABLISHED IN THE ROOT SYSTEM

Supposing the fungus succeeds in reaching the root crown in its course down the stem, it is known from evidence presented previously that it travels along the root and readily makes its way into the buds, which in due time grow up as plants infected secondarily. To what extent does the fungus work back along the root toward the parent plant? Examinations of sections of roots from a number of independently infected plants show that the fungus does invade the roots in both directions, but that the most progress is usually made outwardly. Occasionally, the reverse is true; the diagram of plant 112–C in figure 6 represents the conditions May 22, 1922, in a plant which was inoculated May 5, 1921, when it was a small shoot. The old canes and branches which grew in 1921, are indicated by shaded lines; the new shoots are shown in outline. The infection has ceased to be local and the fungus has established itself firmly in the root as a systemic parasite. All the leaves on the new shoots and leaves at several nodes of the old canes show rust. Buds are pushing out at the base of the old cane 3 inches below the surface of the soil. Six white shoots (C, C′) are developing; a witch's broom is being formed 2 inches farther back along the root. Sections of the root (C, C′), midway between the main stem and the group of small shoots, show mycelium in the medullary rays in the phloem near the cambium, and some in the cortex. Sections of the root 8 inches to the right of C′ show no mycelium.

As no buds or shoots are found on the main root on the other side, one can be rather sure that the fungus in this case traveled back up the root instead of outward, as might be expected. The fact that the root 8 inches distant did not carry mycelium is positive proof that 112–C was primarily infected by sporidia. There is no apparent reason why the fungus might not continue its growth back toward the parent, and thus a parent plant system might become infected through the rust from its root shoots at some distance away. As one finds, then, two infected plants connected by a root carrying the fungus, it is impossible to determine with certainty after the second year which plant was the one primarily infected.

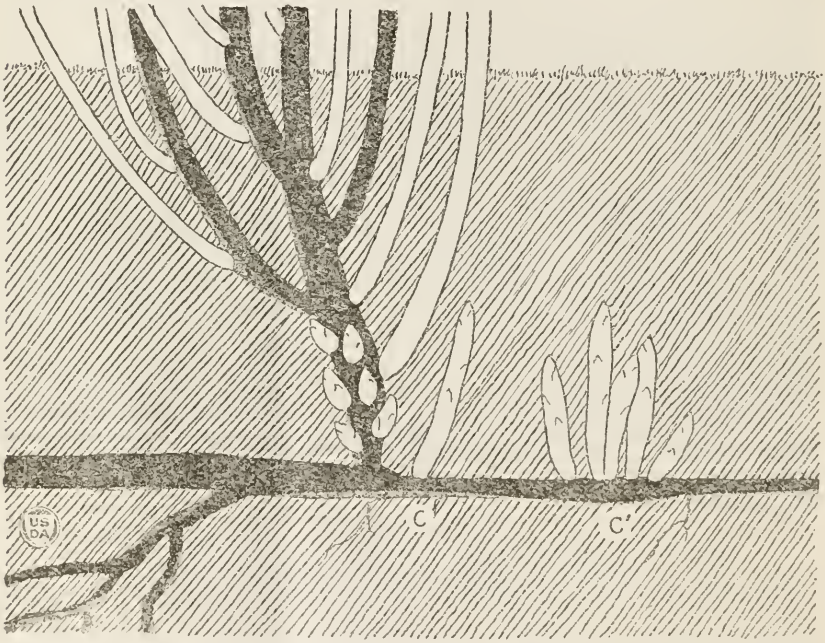

FIG. 6.—Parasite invades root from stem. Diagram of basal part of plant 112 C. Shaded parts of stem represent 1921 growth; branches unshaded, 1922 growth. Mycelium found in cambium, phloem, and cortex of root between C and C′. No mycelium in root 8 inches to the right of C′. See text.

Where one finds in nature several infected plants attached to the same root runner, sections will usually show hyphae in the connecting root. While the writer has not happened to find in nature cases where no mycelium was present, doubtless independent infections of two or three plants which are derived from the same root runner do occur here also. The rust which occurs on young basal shoots of old plants undoubtedly fails in many cases to invade a horizontal root runner and would thus be unable to reach other plants that had arisen in the same manner. The root system of an infected wild high bush blackberry growing in sandy soil was dug up April 13, 1921. It was found that a rusted plant was attached to a root runner 18 feet distant from the original plant which was now dead and decayed. Its large root, 1½ inches in diameter, however, was still alive. Between these two plants there were two small plants not infected, attached to the same root. Passing out in the other direction from the parent, the root runner was followed 12 feet farther.

Sections of this runner at various points in 30 feet of its course showed no mycelium, and as the only rusted plant connected with this long root was the one farthest from the parent, it is clear that the fungus was in this case unable to go back along the root to any appreciable distance, the mycelium having made but little progress beyond the root crown in either direction.

PRIMARY INFECTION SOMETIMES MANIFESTED ONLY THROUGH ROOT SHOOTS

Attention is called to a type of infection the origin of which is not perfectly clear. The plant, 357 A, appears to have been originally infected so near the base of the shoot that the fungus soon reached the horizontal root, where a slight fusiform enlargement 2 or 3 inches long was developed. The shoot grew into a normal cane which showed no rusted leaves May 5, 1922. Instead, three new shoots, thoroughly infected, had grown up from the root a short distance from the old cane (fig. 7). The buds at the base of this cane show that the fungus was

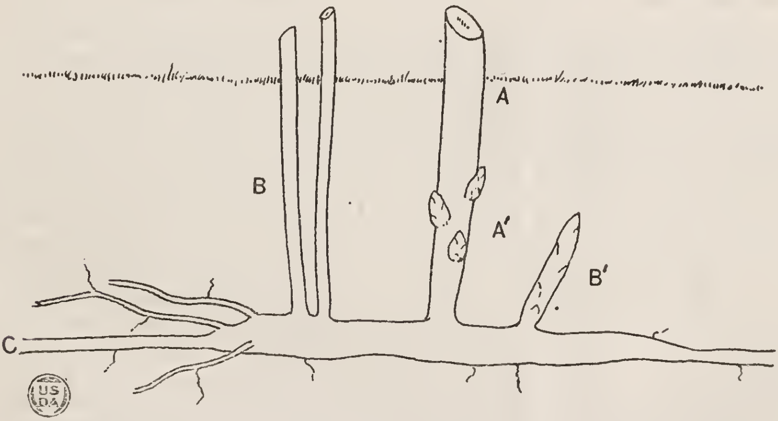

Fig. 7.—Early invasion of root by mycelium from a point originally somewhere near A′ results in root infection stimulating growth of shoots B, B′ Old cane, A, is normal except for buds, A′. which are an indication of the presence of hyphae in that part of the cane.

present in that part which was beneath the soil. The buds would probably have remained dormant, or at least developed only late in the season. The growth of infected shoots on the root at each side of the old cane is against the supposition that the fungus entered the root first through some slight wound, then traveled up the basal part of the cane. The root had given rise in the course of 4 feet to five separate normal shoots, so there is no reason to doubt that the rust now present came as the result of sowing sporidia. A clear-cut case of direct root infection will be described later.

NEW SHOOTS FROM OLD CROWNS SUSCEPTIBLE TO INFECTION

The cases of artificial infection previously described in this paper were of plants which in 1921 were shoots from root runners. In cultivation it is the practice to destroy most of these root shoots which spring up between the rows or between the hills. There is no attempt to destroy the old crown; thus new canes must arise year after year from parts

closely connected with the original crown. Can these new shoots be infected in such a way that the rust becomes established in a plant, the underground parts of which may be several years old? Plant No. 24 (see p. 225), was derived from a wild blackberry crown, potted February 14, 1921. The shoot was infected so near its top that the fungus was unable to reach the underground organs, whereas, if the parasite had entered the shoot when it was just emerging from the soil or at its lowest nodes, hyphae could have penetrated into the underground parts and become firmly established. In some of our experiments shoots from the old underground stem were sprayed with sporidia along with the root shoots. In two instances these shoots became infected. The infection of old crowns, however, by the rust is probably a rare occurrence in nature. When this occurs there will be for several years a number of healthy canes in this hill with only one or two canes showing rust, whereas if the nursary stock or a root shoot is infected the hill derived from either will be worthless from the beginning.

MYCELIUM FROM A PRIMARY INFECTION IN THE GROWING REGION OF THE CANE

Emphasis has been laid upon the point that, in a large percentage of cases observed in our work, the mycelium developing after an inoculation of shoots with sporidia does not travel to any extent up the cane as it grows, but tends to make its way downward into the underground organs, with the result that in the following spring, leaves on new shoots from the base of the cane originally infected and leaves at a few of the basal nodes will develop aecidia, while the cane throughout its upper portion is perfectly normal, bearing blossoms. No mycelium will be found in any of its tissues, except, of course, in the immediate region of the lower rust-bearing leaves. A few notable exceptions to this general rule have been observed. Old canes or branches clearly proved to have been infected as the result of inoculation showed symptoms of having been systemically infected throughout their entire length. Sections of the canes showed mycelium confined to the pith, except at the nodes, just as in canes secondarily infected. Their appearance is quite characteristic, owing to the proliferating terminal and axial buds. Why the fungus behaves in this exceptional way is not clear. No. 135 may be taken as an example of this type of infection. The plant originated as a root shoot from a Kittatinny blackberry 2 feet away. The parent plant has shown no rust for three years. The shoot was about 1 foot high when inoculated with aecidiospores May 23, 1921. On September 28 it was noted that a branch from the base of the plant appeared somewhat abnormal, as though it might be infected. On April 28, the following spring, pycnia appeared on the leaves of the new shoots. On May 8 the infected plant, including the entire root system back to the parent plant, was dug up. The plant now consisted of one large 1921 cane which had been broken off 14 inches above the soil during the preceding summer. Leaves at the basal nodes were rusted and three large new shoots from its base had developed; to this extent the infection appeared to be typical. There was, however, one large 1921 cane (a branch from the lowest node of the cane originally inoculated) which showed peculiarities in that several of its axial buds had been stimulated to grow out into new shoots (Pl. 6, b) and the terminal bud of the 1921 cane had also grown out into a long new shoot, a. This certainly is exceptional behavior for our blackberry, whose

growth is indefinite, the tip ends of the cane being regularly killed at the end of the season. This proliferating tip end was about a foot long. Sections of this 1921 cane 18 inches back of the tip at c showed mycelium only in the pith. The effect of the proliferating shoots along the cane may be seen by the partial second annular ring of wood being formed (Pl. 2, C, y). At the swollen base (Pl. 6, a′) of the 1922 portion, mycelium was also found in the phloem and cortex leading to leaves from this swollen region. Sections of the new growth 2 inches above (a′) showed mycelium only in the pith. Until extended study has been made of the actual point of penetration of the host by the fungus it will be impossible to say definitely when the parasite could have been established in the growing point of this old branch. Mycelium showed only in the pith, except at the nodes where it could be found in the phloem or in the cortex even.

That the original point of infection is not ordinarily the terminal growing region of the shoot is clear from the large percentage of artificially infected canes which show no mycelium in the pith at any point, and none at all in the cane along the greater portion of the upper region. It is possible that where the cane is broken off soon after infection, the axial buds in which the fungus has become established may be stimulated to grow out vigorously, and if the fungus were in the fundamental tissue of the growing region, it would later appear in the pith after tissue differentiation. The writer has observed no case where the main shoot inoculated behaved as though the terminal bud had been attacked, though this no doubt occurs occasionally despite the protection of the growing region, by overlapping leaf primordia. The rapidity with which young canes grow up undoubtedly enables them to outstrip the fungus, which at first seems to grow only slowly, so the mycelium would be left behind in the cortical and phloem regions. Hyphae then make their way slowly up the stem for a short distance and more rapidly downward into the underground stem, and even into the roots. Here the presence of the parasite stimulates the formation of buds, the growing regions of which will be invaded by the hyphae, and as these buds develop into the new shoots the following spring, the fungus, now firmly established, grows upward rapidly and will be found in the pith even at the tip of the new cane.

The writer's experiments show that infection can take place when shoots are several inches high, but in such cases the fungus rarely grows downward with sufficient rapidity in northern latitudes to become firmly established in the perennial underground organs. The younger the shoot inoculated or the nearer the root the infection occurs, the more certain is the fungus to become systemic the following year.

If plants are grown in flats in the greenhouse where the canes are prevented from becoming dormant until late, infection of large shoots at two or three nodes spreads rapidly through the cambium and phloem upward nearly the full length of the stem, and downward well into the root system. Such infections are thoroughly systemic, but no mycelium will be found in the central pith, and there will be no proliferating terminal bud. In the southernmost states where the growing season for blackberries is almost continuous one would expect this type of primary infection to be common in nature.

EFFECT OF INJURY TO THE GROWING POINT

Very frequently when the hypodermic needle is used to inoculate the growing point of a shoot, the injury leads to the death of the end punctured. Sometimes when the soil is being removed from around the root sprouts, the ends will be broken off so that the effect on their future development is practically the same. A new shoot immediately grows out from an axial bud below. The following spring the cane into which the shoot develops will have a short right-angled bend at the point where the scar tissue healed over the end-portion which had died. The plant shown in Plate 4, B, a, also illustrates this feature. On May 5, 1921, the soil was dug up around a plant of the Iceberg variety so that three shoots about 2 inches long were exposed. No. 112 A was inoculated by injecting sporidia into the growing point and spraying them over the surface; aecidiospores were also dusted over the young plant, with the hope that one or the other method might result in infection. No. 112 B and 112 C were merely sprayed with sporidia. The growing point of the first plant which had been injured died, and three new shoots grew out soon after from axial buds below. One of these became the leader and also gave rise to a secondary branch. The leader grew to be 5 feet high and blossomed normally the next year. Leaves at the lower nodes bore the orange-rust May 3, 1922. Four new shoots, systemically infected, now arose from the base of the main cane, but its 1921 branch, as well as the other two canes that were developed in 1921, as noted, showed no rust. The mycelium apparently had not penetrated the horizontal root to any extent, as there were no shoots or buds on the root such as one finds when it is infected. It was found that this root had also given rise in 1921 to the shoot 112 B, mentioned above. Infection resulted from spraying sporidia on the shoot. In May, 1922, it consisted of a single old cane bearing an abundance of blossoms, and the leaves only at the lowest nodes were rusted. New shoots, bearing pycnia were springing up from that part of the cane beneath the soil.

When plants A and B are compared it is seen that the result of killing the growing tip of the inoculated shoot in plant A was simply to stimulate the axial buds to grow into new branches at once, but the type of infection in this case was not altered thereby. Anything that stimulates the development of new and therefore more susceptible shoots or branches from time to time throughout the period of spore dispersal, which varies from two weeks to a month or over, increases the chances of infection proportionately. Very striking effects of accidental layering of *Rubus enslenii* in a neglected cemetery at Winston-Salem, N. C., were observed in April, 1922. A number of graves had been dug the preceding year and the dirt thrown out over these wild dewberry vines. The following spring practically every shoot that was found growing through the covering of dirt from the excavations was rusted. Very few rusted plants were found in this cemetery where the vines had not been disturbed or covered.

ROOTS SUSCEPTIBLE TO INFECTION

One frequently finds orange-rust in hedge rows along the margins of cultivated fields, along embankments, or in pastures, where injury to the canes and roots is very likely to occur. This has suggested the possibility that the blackberry may also be primarily infected through its roots which become exposed through cultivation or otherwise. A few cases of infection were found in the writer's work which were difficult

to explain on any other basis because of the absence of an old cane at the point where infected shoots were then growing. More careful search at such times usually showed the stub-end or scar of a cane which had been cut or borken off accidently or which had died the previous summer. In the absence of such structures through which the parasite might have entered the root, one naturally suspects that the mycelium has traveled along the root runner from some other infected plant. If such a possibility is precluded by an examination of sections, one would have to consider the alternative—direct root infection, which could occur only on exposed roots and before the cork covering had been laid down, or through wounds. For example, in one case it was found that the manner of infection could not be determined at first because of the absence of any remains of, or evidence of, the existence of the orginally infected shoot. Several shoots of the Mercereau (No. 352) blackberry were sprayed with sporidia April 25, 1921. No inoculating chamber was used. On May 8, 1920, aecidia were found on three plants, No. 352 A and B showing the ordinary type of primary infection. Old canes which were rather small and somewhat dwarfed were present and infected shoots were growing from the base of each. There was no old cane nor any trace of a stub or scar showing where one might have been, in connection with plant No. 352 C, the third plant infected. Two new shoots, both infected, and about a dozen buds were growing from a horizontal root where it was somewhat enlarged (Pl. 7, A). Thirty-two inches from these shoots the basal end of an old cane b, was found. The small root runner was followed 14 inches farther where it was attached to a larger root having several branches, c. This root had its origin 18 inches away in a large perfectly normal cane now in blossom. Sections of the horizontal root were made at points as follows: One foot from the parent plant; c_2, 6 inches beyond the stub of the 1921 cane; c_3, 3 inches from the first rusted shoot which was sectioned at c_4. No mycelium was found in the root sections, proving conclusively that the infection had not been carried over from the parent plant. Hyphae were found in the pith only of the infected shoot, c_4, as was expected. It was observed that the second shoot was somewhat woody at its base and had a number of scale leaves about one inch from the point of attachment, indicating that this part of the shoot had been formed the previous summer. This could not have been the shoot originally inoculated and the one which had remained dormant after infection.

It has been noticed that where shoots of the witch's broom type grow up early in the spring they are often woody at the base, which is covered with scale leaves. The buds are forced out the preceding autumn, lie dormant over winter, being protected by a covering of soil, and push up early in the spring. Such conditions are easily found in naturally infected plants. In the case being considered, it is possible that the root had been exposed about the time the inoculation of other shoots had been made and the fungus entered the root probably through a wound. Since shoots devoid of chlorophyll can be most easily infected and since the fungus can thrive in underground parts, there is no evident reason why very young roots may not be directly infected through unprotected epidermis or through wounds extending to the cambium. Evidence in support of such a probability was found in connection with No. 357E, a photograph of which is shown in Plate 7, B. The parent stock, planted in 1920, still bore large healthy canes in 1922. One other old cane

free from rust May 5, 1922, was found attached to the horizontal root 6 inches from the parent crown. Sixteen inches beyond, infected shoots were vigorously developing. Sections of the root at x, y, and z were made but no hyphae were present at these points. No old cane nor any remains of such could be found among the new shoots. There was, however, a very definite scar tissue partially covering an old wound, at w, about an inch long. The root at this point was, when dug, not buried over one-half an inch in the soil, as is clear from the photograph. I do not doubt in the least that the root was originally infected in this wounded tissue.

INFECTION OF BLACKBERRIES IN THE GREENHOUSE

For reasons previously stated, very little work was done in 1921 in attempting to infect blackberries in the greenhouse. As the rust can certainly remain alive in the crown and roots of a plant at least a year without appearing in the canes, one is not certain that his plant, brought in from nature, may not be infected. Such plants should be observed two or three years before being used for experimental purposes, no matter how many controls are used. On the other hand, by careful investigation of the distribution of the mycelium in various structures of an infected plant, one need not remain in doubt, in most cases, as to the time his plant became infected. For example, if mycelium is found in the pith of an old cane whose leaves are rusted the spring following inoculation, it is pretty good evidence that the fungus was present in the plant at the beginning, but if hyphae are found in the phloem, near the outer ends of the medullary rays, or along the cambium or in the cortex, but not in the pith, this will be very good evidence that this cane was infected through inoculation with spores. Only in rare cases does the rust establish itself in the growing point at the time of primary infection. This does sometimes happen, as noted elsewhere, but such cases are very characteristic in their growth the spring following. In the spring of 1922, a number of blackberry shoots growing in "flats" were inoculated with the short-cycled rust. As sections of canes which developed rust the following spring showed no hyphae in the central pith, it is evident that the infections were primary and the result of the inoculation.

PRIMARY AND SECONDARY SYSTEMIC INFECTIONS CONTRASTED

Plants set out in the benches or in large "flats" make root shoots at some distance from the parent plant, but they are not very satisfactory for these experiments. Twelve root shoots of rust-free Kittatinny blackberries were planted in "flats" in the greenhouse April 13, 1922. By May 29 new canes had grown to be from 6 inches to 2 feet high. In order to determine whether canes of such a size could be infected systemically, they were sprayed at this time with aecidiospores of the short-cycled rust from a wild blackberry, and kept in the damp chamber three days. Having been overwintered in the cold frame, they were brought back to the greenhouse March 10. Leaves soon appeared on the old canes which had been inoculated, as noted, the previous year, and new shoots began to grow up from the base of the canes. For at least two weeks all leaves formed were perfectly normal, dark green, hairy on both sides, and not dwarfed. There was nothing abnormal in the appearance of the canes. About March 24 the latest leaves just unfolding at certain nodes became yellowish-green at the margins, which were more finely lobed and wrin-

kled, and devoid of hairs. The petioles tended to elongate abnormally. Some of the axial buds were also beginning to proliferate. The distribution of stomata on the leaves which appeared to be normal and on those showing signs of infection was studied. Practically the same number of stomata was found on the underside of both kinds of leaves. There were only a very few on the dorsal side of the normal leaves, occurring mostly at the tips of the serrations. On the other hand stomata were thickly scattered over the upper epidermis of the abnormal leaves where they showed yellowing. Although no pycnia or aecia are present, the occurrence of large numbers of stomata on the upper side of a blackberry leaf is proof that the orange-rust hyphae have invaded the leaf. The first aecidia appeared April 3. If spermogonia were ever formed on these rusted plants they must have been "vestigial."

The writer had about the same number of systemically infected plants growing under similar conditions at the time, so it was possible to compare blackberries in which the infection had been of long standing, at least two years, with those showing the rust for the first time. The canes of the former were less angular and showed fewer thorns. Pycnia appeared all over the leaves as soon as they began to unfold. Every leaf at each node was dwarfed and yellowish from the start. Stomata were pretty evenly and thickly distributed over the upper surface between the pycnia. Here also it was found that there were about the same number of stomata on the underside of the normal and of the infected leaves per unit area. The total number of stomata per unit area was estimated to be fully 50 per cent greater on the infected leaves. Normal leaves from certain uninfected canes of this plant showed only a few stomata on the upper surface of the tips of the serrations.

A further discussion of the effect of the orange-rusts on the development of stomata is being published in another paper. Unless one were very familiar with the appearance of various types of primary infections, he would have passed over the writer's plants which had been infected the preceding summer because no spermogonia could be found. At the same time the plants that were secondarily infected showed the characteristic symptoms of having the orange-rust.

SPECIALIZED RACES

Since the long-cycled rust is fairly common on black raspberries in localities where the teleutospores have not been found on the blackberries, the possibility of the existence of strains or biologic races should not be overlooked. Whether the blackberry or dewberry can be infected with aecidiospores from the Gymnoconia on black raspberry or *vice versa* has not been definitely questioned by previous investigators. Morphologically the long-cycled rust on black raspberry has not been found different from the rust on blackberry. The writer has made a few preliminary experiments to see how readily the teleutospore stage could be obtained on one species of host by sowing aecidiospores from another variety. These tests are regarded as merely suggestive.

On May 18, 1921, young leaves of new shoots of wild black raspberries at Bell, Md., were inoculated with aecidiospores from the mountain blackberry. On August 20, several teleuto sori were found on one of the lower leaves of one plant. The plants were well isolated in a hedge row 30 rods from any orange-rust. If leaves on old canes had been present when the inoculation was made, no doubt the infections would have been more abundant. Two hybrid blackberry plants, with predominant mountain

blackberry characters, were inoculated with aecidiospores from a wild "yellow" (black) raspberry May 18, 1921. On July 19 many sori were found on the leaves of young canes of one of them. No sori could be found on other plants of the same variety near by. During the spring of 1922 these experiments were repeated more satisfactorily in the greenhouse. Several wild blackberries were planted on a bench. After they had grown out vigorously they were inoculated on several different days with aecidiospores from black raspberries. Spores were rubbed and sprayed on the underside of young and of old leaves, and infected raspberries bearing aecidia were placed under the same infection tent. Thus the blackberry leaves were frequently covered with spores, and conditions were certainly made favorable for infection. At no time later during the summer could telia be found on any of the leaves, old or young, although leaves of the black raspberry plants which had been set in the same infection tents, as noted, were later covered with sori, and they had not been especially inoculated. Four root shoots from these plants were systemically infected with the short-cycled rust in 1922. Such results might be interpreted to indicate that biologic races occur and that the blackberry is immune to the long-cycled rust from the black raspberry, but of course it would first be necessary to prove this particular species or variety of blackberry to be susceptible to infection by the sporophytic stage of the long-cycled rust from other blackberries. Six Kittatinny blackberries were sprayed on May 29, 1922, with aecidiospores from the black raspberry and six others with aecidiospores from a wild blackberry found at Upper Marlboro, Md. On July 19 a few teleuto sori appeared on leaves of each set, and on August 2 other leaves were found well infected with the rust from both sources, suggesting that the Kittatinny variety is susceptible to infection by the long-cycled rust regardless of whether the rust comes from the black raspberry or from this wild blackberry.

It will require much work to settle definitely the question of biologic races of the orange-rusts owing to the difficulties encountered in systemically infecting a susceptible plant with the gametophytic stage. The difficulties can not be overcome nor the questions answered by working with the telial stage alone, because a plant might be susceptible to infection by the telial stage of a certain strain but not be to its orange-rust stage or *vice versa*. The long-cycled rust occurs on blackberries, dewberries, and black raspberries. Can teleutospores be obtained with equal readiness on the dewberry by sowing sporidia from teleutospores obtained from each of the three types of hosts? In spite of the evidence of his preliminary experiments, the writer can not believe that the answer to each question will be in the affirmative.

During the fall of 1920, Mr. George Darrow had some plants of *Rubus canadensis*, the mountain blackberry, sent from Phillips, Me., to Bell, Md., for breeding experiments. They were planted in a plot adjacent to several cultivated varieties. In May 1921, these plants appeared to be seriously affected with the long-cycled orange-rust. During July and August large numbers of teleuto sori were found on the leaves of these mountain blackberries and spores were not rare on the leaves of the Ward, Joy, Mercereau, and the loganberry, showing that in the vicinity of Washington, D. C., the sporophytic stage spreads naturally from the mountain blackberry to several of our well-known varieties of other species. There are at Arlington Farm, Va., some loganberries adjacent to a wild dewberry originally obtained from Phillips, Me., when in an

infected condition. On June 24, 1921, leaves of one of the loganberries whose vines intermingled with those of the infected dewberry, bore large numbers of teleutospores. Another loganberry was inoculated May 23 with aecidiospores from this dewberry. On July 21, teleuto sori were found on several leaves of the loganberry. One of these loganberries had also become naturally infected with the "Kunkelia." It will be interesting to learn whether this variety is susceptible to the gametophytic as well as to the sporophytic stage of the Gymnoconia. It does not follow that a species or horticultural variety is susceptible to the orange-rust stage simply because its leaves can be made to mature teleutospores. Such an example of close heteroecism has not as yet been described, although it may occur. Several plants of the Iceberg variety of blackberry were inoculated with sporidia from aecidiospores from the wild dewberry. Although shoots chosen for this work were in the best condition for infection at the time, no positive results were obtained. This variety is very susceptible to the rust from other blackberries, and probably is not altogether resistant to dewberry rust. More work will be necessary to prove this point.

SUSCEPTIBLE VARIETIES

Plants of different varieties of blackberries and raspberries have been inoculated with aecidiospores of the Gymnoconia without obtaining teleutospores. Some of these failures have been due undoubtedly to faulty technic rather than to the immunity of the host to the sporophytic stage of the long-cycled rust. The Lucretia dewberry certainly approaches complete immunity in North Carolina. The writer has never seen orange-rust on this variety, although no attempts were made in the first experimental work to infect it. Inoculations of the Iceberg, Crystal White, Kittatinny, Mercereau, Blowers, Ancient Briton, and the Crandall with the short-cycled form resulted in such success that the failure to infect a variety of blackberry received from a nursery under the name of "Lawton" suggests that this form, whatever may be its true name, is probably immune to the short-cycled rust. This variety formed so many root shoots in April, 1921, that it was especially chosen for infection experiments without anything being known about its susceptibility. Seven separate experiments in which about 30 shoots were inoculated in various ways were carried out. Since the conditions for infection were fully as favorable as were those which resulted in such marked success with the Kittatinny plants which grew near this "Lawton" variety, it certainly must be very resistant. Several plants of the McDonald were inoculated in the open, but no infection resulted. The attempts to infect the "Lawton" were repeated in 1922 with still greater care but no infections were obtained. The Snyder blackberry also proved to be very resistant. The Taylor, Blowers, and Ancient Briton inoculated at the same time were easily infected.

Fifty separate sowings of sporidia were made on root shoots of cultivated varieties of blackberries in nature in 1921. From 1 to 10 shoots in each case were covered by the infection chamber, so on an average 3 or 4 shoots were inoculated each time. Every attempt to infect the variety, Lawton, as noted above, resulted in failure. Only one plant of the "Eldorado" variety was infected although a number of shoots were sprayed with sporidia. The Eldorado is said to be resistant. At least one shoot of the varieties mentioned was infected in each of the

other 48 trials. The number infected in each case is as follows: Crandall, 8; Iceberg, 23; Crystal White, 26; Kittatinny, 30; and Mercereau, 38. The variety which was shipped to the writer as the Mercereau appears to be the most susceptible. Many infections of this variety were obtained merely by spraying shoots or root sprouts with sporidia without using an artificial humidifier. The results obtained in later experiments, 1922–23, but not included in this summary and not given in the table, furnish further evidence that our commercial varieties of blackberry vary exceedingly in the degree to which they are susceptible to orange-rusts.

The Oregon Evergreen (Black Diamond) blackberry is said to be very resistant to orange-rust, yet the writer found that it could be easily infected by sowing aecidiospores from wild blackberry on its fully expanded leaves, teleutospores developing in about six weeks. This variety, however, may be at the same time very resistant to the gametophytic stage of the long-cycled rust, and to the short-cycled form as well. The southern dewberry, *Rubus enslenii*, which is subject to attack by the short-cycled orange-rust, was readily infected by sowing aecidiospores of the long-cycled Gymnoconia from black raspberry on the leaves. So far as the writer knows, the aecidial stage of this form has not been found on *Rubus enslenii*.

ORANGE-RUST AECIDIA ON CANES AND FLOWERS

If the mycelium, lodged in the perennial underground parts of a blackberry, penetrates a shoot bud and grows up with the cane, this cane, or any part of it bearing hyphae, does not blossom. The localized primary infections described previously are not included in this category, because the mycelium would be unable to reach the growing point and thus grow up with the cane. What at first appeared to be an exception to this rule was noted at Salem, N. C., where several plants of the wild dewberry, *Rubus enslenii*, infected with the short-cycled orange-rust, bore aecidia on the fruit branches, leaf stalks, and even on the calyx of flowers which were being formed in the normal fashion. These were cases of primary infection by sporidia; this was proved by a study of the distribution of the mycelium.

WITCHES' BROOMS ON OLD CANES

The ultimate effect of the orange-rust on most plants if not disturbed by pruning is to cause the canes to become dwarfed or spindling, and to grow out in large numbers from the infected crown. Such brooms should be distinguished from the small ones found at the nodes on certain old canes. It has been thought by some that these distortions are also caused by the rust which is so often found on the leaves at these points. The "double blossom" fungus appears to be able to attack the axial buds of canes infected with the orange-rust so that in the spring both parasites are found together, but it is the "double blossom" fungus that causes the formation of the brooms. These malformations are most common in North Carolina and Virginia, and do not often occur in northern regions on plants infected with the orange-rust, merely because of the absence of the "double blossom" fungus. It is the parasite that lives for the most part on the surface of the organs rather than the one found within the tissues, that stimulates excessive development of buds in old canes.

DISTRIBUTION OF THE ORANGE-RUSTS

The long-cycled Gymnoconia is the only orange-rust known in Europe and Asia. When it was discovered that there were two of these rusts in North America it was said that the short-cycled form was southern in its distribution and the long-cycled strictly northern, the former being the rust so destructive to the blackberries and dewberries grown commercially. It is now known that it is no longer necessary to make pilgrimages to Bartlett, N. H., for the Gymnoconia, because this rust thrives wherever the black raspberry, *Rubus occidentalis*, or its susceptible horticultural varieties may grow. The writer has reported (5) that the rust is common on blackberries near Washington, D. C., and at Old Fort, N. C. He has since found it in abundance on blackberries at Salem, N. C., the type locality of "Kunkelia *nitens*" and at Cornelia, Ga. Germination tests reported in a letter to the author by Dr. Dosdall, mycologist at the Minnesota Agricultural Experimental Station, show that the Gymnoconia is probably very common in that State. No doubt it will be found wherever susceptible blackberries grow. The short-cycled rust, having been recently derived from the other form, is less widely known. Its spread undoubtedly has been accelerated by commercial shipments of diseased nursery stock from one part of this country to another. The temperature ranges through which the Gymnoconia thrives are not different from those suitable for the short-cycled rust, once each is established in its host. One reason why the Gymnoconia follows the black raspberry north and south and why this host is not attacked, at least to any extent, by the short-cycled rust, is that the teleutospores mature at the same time that susceptible tip plants are being developed.

CONTROL OF THE ORANGE-RUSTS

Methods by which the orange-rusts can be eradicated have been suggested in connection with the discussions of the infection experiments. It was pointed out that a blackberry can be freed from the orange-rust very easily if the task is undertaken soon after the primary infection becomes manifest. The mere snapping off of the infected cane at the point of attachment to the root will suffice in many cases. When a number of shoots in the form of a witch's broom are found, it usually indicates that the fungus has invaded the root or its crown; it will then be necessary to destroy this part of the root also. If the primary infection, however, is allowed to spread to the crown and root system the second year, so that new shoots are systemically and secondarily infected, the whole plant must be dug up, care being taken to include the roots for some distance.

It has been shown that it is of the greatest importance to begin a planting with rust-free nursery stock. If the black raspberry to be used has been propagated by rooting the tips of canes one may be reasonably sure of getting some infected plants—that is, if the telial stage of the Gymnoconia is present in the nursery. Whether the tips of canes can be made to root early enough to avoid infection by the first sporidia from teleutospores or late enough so that no buds or shoots that can be infected are formed before the frosts, are problems which will require further investigation. If nurserymen will destroy all infected canes before aecidiospores are shed, there will be no teleutospores in their propagating fields, and it follows that their tip-plants will not be infected when sent to the grower.

So far as controlling the short-cycled rust in the cultivated blackberry is concerned, the writer's experimental work is showing that it is perfectly practicable with a small amount of labor to prevent the spread of the rust. Primary infections by spores occur comparatively rarely in nature; thus, if one observes proper care for a period of two or three weeks in early spring as soon as the first leaves appear, he can readily detect and destroy rusted canes before the mycelium has spread far into the underground perennial structures and before the spores are shed.

The eradication of all rust from a field of blackberries where the disease has been of long standing would be a more difficult undertaking. In New Jersey and in other states one can find fields where from 25 per cent to 75 per cent or more of the plants are infected. Such fields should be planted to some other crop unless the grower is willing to follow up and destroy all roots connected with the rusted plants.

The work at Arlington, Va., reported above, affords a very good illustration of the efficacy of removing infected plants as soon as they show rust for the first time. The writer had about 130 cases of primary infection; wherever the rusted canes were pulled up so as to include all parts of the root runner which showed signs of infection, no rust appeared in 1923. In several cases where it was recorded that undoubtedly pieces of roots bearing mycelium were left in the soil, rusted plants showed in 1923.

Probably one reason why infections by sporidia of the short-cycled rust are comparatively so rare when one considers the vast number of aecidiospores that are matured, is that these spores are rather waxy and therefore, like waxy pollen, are not blown for any great distance by the wind. It would not be good practice, in any event, to allow a rusted plant to remain in the field or to encourage a luxuriant growth of rusted wild blackberries in the vicinity of susceptible cultivated varieties. Two or three days of wet weather at the time new shoots are springing up would certainly result in a further spread of the disease by spores from plants near by. The rust can pass over just as easily from a wild blackberry as from a cultivated variety.

SUMMARY

(1) A study has been made of the distribution of the gametophytic mycelium of the short-cycled orange rust in the blackberry and dewberry, and of the mycelium of the long-cycled rust in the blackberry, dewberry, and black raspberry. In the canes of the blackberry in which either rust has become firmly established as a perennial parasite, hyphae are mostly confined to the central pith and to the fundamental tissue of the growing regions. At the nodes traces of mycelium are sometimes found along the rays in the wood and in the cambium and phloem. Hyphae penetrate the roots very extensively, following the cambium and sieve tubes of the root runner many feet. The cortex is also attcked. Very little mycelium is present in the woody tissue; there is no central pith in the root. New plants arising from the infected root runners will be infected. The spread of the rust from plant to plant in nature occurs frequently through the connecting roots. Mycelium invades the roots and rootlets of infected dewberries very generally but does not follow a root to any great distance. The rust is carried to new plants formed at the rooting nodes by the invasion of these sprouts by hyphae from the vines where the mycelium is distributed in about the same way that it is in the canes of blackberries.

(2) The roots and rootlets of the black raspberry are attacked by the hyphae, mycelium being found not only along the rays but very generally in the wood ring and along the cambium and phloem. As in the dewberry, the mycelium does not follow the roots for very great distances. Hyphae have been found in roots 8 or 10 inches long.

(3) Canes of a thoroughly infected black raspberry do not root at the tips very readily; therefore the long-cycled rust is not so often spread vegetatively to tip plants from an infected parent. The infection of very young tip plants by sporidia from teleutospores largely accounts for the appearance of the rust on new plants. The wild raspberry, *Rubus occidentalis*, and the horticultural varieties, Plum Farmer and Cumberland, were infected by laying black raspberry leaves bearing teleutospores over rooting tips of stolons and maintaining suitable moisture conditions. Infections of the black raspberry also occurred when the teleutospores were taken from blackberry leaves.

(4) Susceptible blackberries can be infected with the short-cycled rust by sowing the sporidia formed on promycelia from germinating aecidiospores on young root shoots; 150 separate primary infections were made in this way. If a blackberry cane has been primarily infected by sowing sporidia, the hyphae of the rust will be found in most cases to be confined to the cambium and phloem tissues. Only rarely do hyphae become established in the tip of an inoculated shoot and grow up with the cane as does the mycelium in a cane from an infected hill. Localized gametophytic primary infections which do not become permanently established sometimes occur, especially if the young cane is several inches high when inoculated. A cane primarily infected usually blossoms normally except at the infected nodes, but canes arising from an infected hill and having mycelium in the growing regions from the beginning do not blossom. Certain nodes may happen to escape invasions by hyphae; in this case blossoms will develop.

(5) Measures for controlling the orange rusts are suggested, emphasis being laid on a thorough inspection of nursery stock for at least one month after planting, and the complete eradication of plants showing rust. Primary infections of blackberry from sporidia are very characteristic and at first do not involve the whole plant and its root system. A limited amount of the root directly connected with the infected cane will usually have been invaded by the hyphae from above, so the destruction of this old cane with a few inches of the root will be sufficient. If such canes are allowed to grow, the parasite soon becomes established in the root crown and it will then be necessary to uproot and destroy the whole plant. As a matter of safety in every case the infected canes and all attached roots should be destroyed.

(6) The infection experiments prove: (a) That the short-cycled rust on wild blackberry can infect such cultivated varieties as the Kittatinny, Iceberg, Mercereau, Crandall, Taylor, Blowers, Ancient Briton, etc.; (b) that the sporophytic stage of the Gymnoconia will go over from the mountain blackberry, *Rubus canadensis*, to such varieties as the Ward, Taylor, Mercereau, and Loganberry, and that teleutospores can be obtained on leaves of certain blackberries and dewberries by sowing aecidiospores from the black raspberry, which can in turn be likewise infected by sowing aecidiospores from the blackberry; (c) that the black raspberry can also be systemically infected with sporidia from teleutospores of the long-cycled rust on blackberry.

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We have no reason to suspect that the rust on the wild blackberries is in any way unlike that found on the cultivated blackberries, or that forms of the long-cycled rust on the blackberry and on the black raspberry are at all different biologically, except that certain strains may prove to be more vigorous in their parasitism. As between the systemic stage of the short-cycled and that of the long-cycled rust as they occur on different types of host plants, and as between these and the teleutosporic or sporophytic stage of the Gymnoconia as it is found on blackberry, dewberry and the black raspberry, it must be expected that considerable difference will be found in the readiness with which particular hosts can be infected. Later infection experiments tend to show that the Iceberg blackberry which is very susceptible to attack by the short-cycled rust from the blackberry, is very resistant to the rust from the wild dewberry. This short-cycled dewberry rust may be somewhat different biologically.

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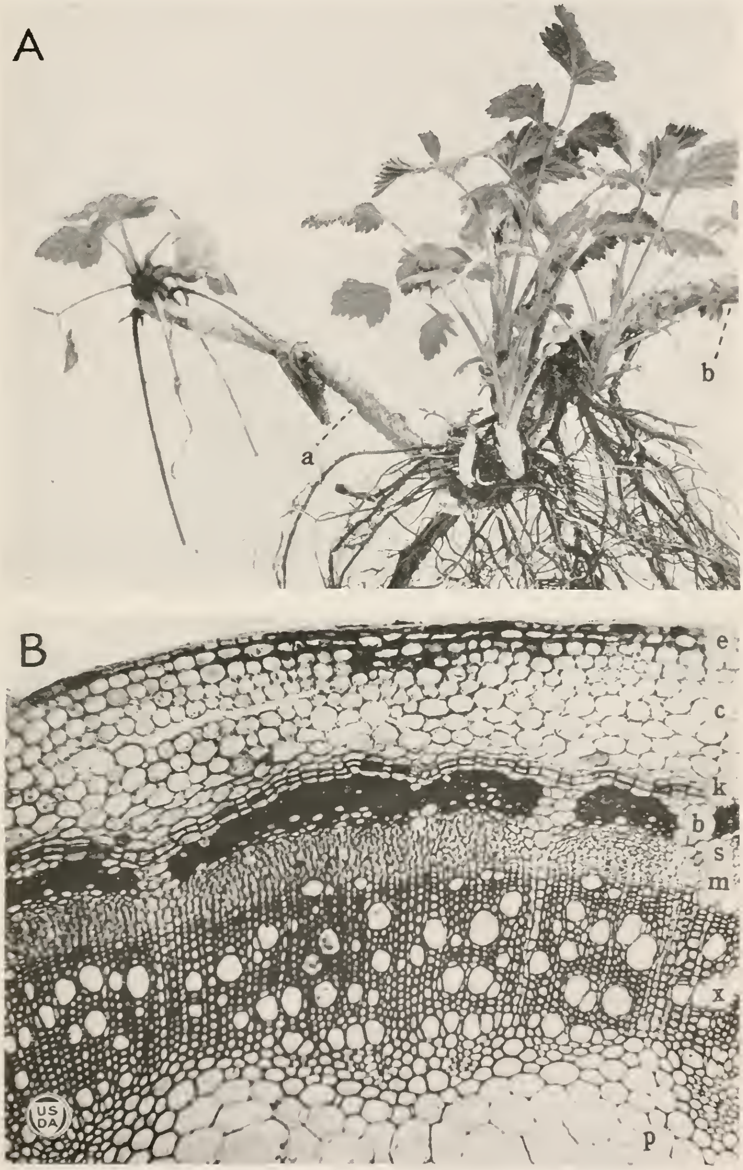
2421

PLATE 1

Rubus occidentalis, black raspberry, infected with the long-cycled orange-rust.

A.—Tip plant which was infected with sporidia of the Gymnoconia in August, 1921; photographed March, 1922.

B.—Section through the cane at b in A: p, pith; x, wood ring or xylem; m, cambium; s, sieve tubes; b. hard bast or stercome (tissues s and b constituting the phloem) k, cork cambium; c, cortex; e, epidermis. Sections of the stolon at a and b in A showed no hyphae; midway between a and b there was an abundance of mycelium in the cambium and phloem, but none in the pith. Sections through the base of each infected shoot revealed hyphae in the pith, phloem, and cortex. See figure 1 for further details.


A
a
b
B
e
c
k
b
s
m
x
p
USDA

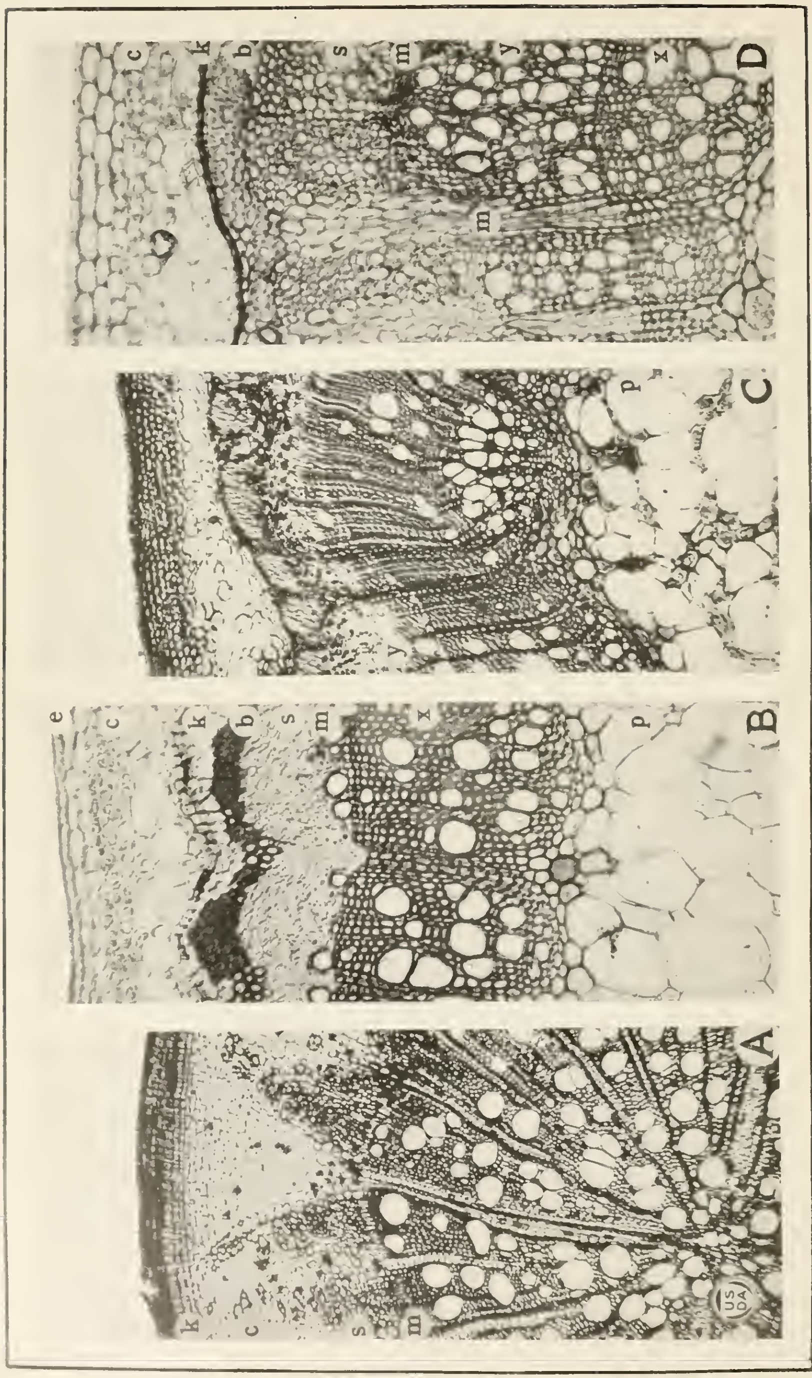

A
B
C
D

PLATE 2

The small letters have the following significance: p, pith; x, xylem or wood ring, y, second wood ring; m, cambium; s, sieve tubes; b, hard bast or stereome; k, cork cambium or phellogen; c, cortex; e, epidermis.

A.—Cross section of root of blackberry.

B.—Cross section of 2-year-old blackberry cane. Note only one wood ring.

C.—Section of infected cane of blackberry, No. 135, at c in Plate 6, A, just below the proliferating branch, b. Note a partial second wood ring, y. Mycelium was found only in the pith.

D.—Section below a primarily infected branch of blackberry cane, No. 24. The mycelium grew down into the stem along the cambium which was stimulated to form a new ring of abnormal wood and additional phloem. In this section hyphae were ound all through the wood in the second ring, y, and in the rays and phloem.

PLATE 3

A.—Cane and roots of a Crystal White blackberry, No. 342 D, showing localized primary infections on two branches, k and l, of the old cane, uninfected tip ends of cane, and branches cut off. No hyphae were found in sections of the cane at the base, and none in the roots at the cut ends shown here.

B.—Localized primary infection at one node of a cane of a Kittatinny blackberry. Such local infections can not become established systemically because the canes die naturally before the fungus can reach the underground perennial parts of the plant. If such infections occurred on plants grown in the greenhouse or in the southernmost states where the growing season is very long, hyphae would very likely be able to reach the crown of the plant before the cane died.


l
k
353
B
A
B


b
a
A
B

2425

PLATE 4

A.—Common type of primary infection of Iceberg blackberry No. 334 F. The cane above is normal, blossoming profusely, and is free from mycelium. Two or three new shoots are arising from the base of the cane. Hyphae have not yet invaded the base of the cane below the ground, but would have soon done so if the cane had not been destroyed at this time.

B.—Iceberg blackberry in which the growing point had been injured at the time the shoot was inoculated. The present main cane now in blossom, b, arose at once from an axial bud. This cane is free from the rust except at its lowest node. Several infected shoots have developed from the basal part of the cane. The fungus has not yet invaded the root.

PLATE 5

A.—Cultivated blackberry the second year after infection. The old canes are stunted and without blossoms. New shoots are in the form of a typical witch's broom. The longer the rust lives in a plant the more numerous and spindling become the canes.

B.—Taylor blackberry with localized primary infections at the nodes showing etiolated shoots. The upper part of the cane is perfectly normal and would have borne fruit. Such an infection will not become constitutional. The mycelium can not reach the root crown before the cane dies naturally.

2426


B
b
c
a'
a
A

2427

PLATE 6

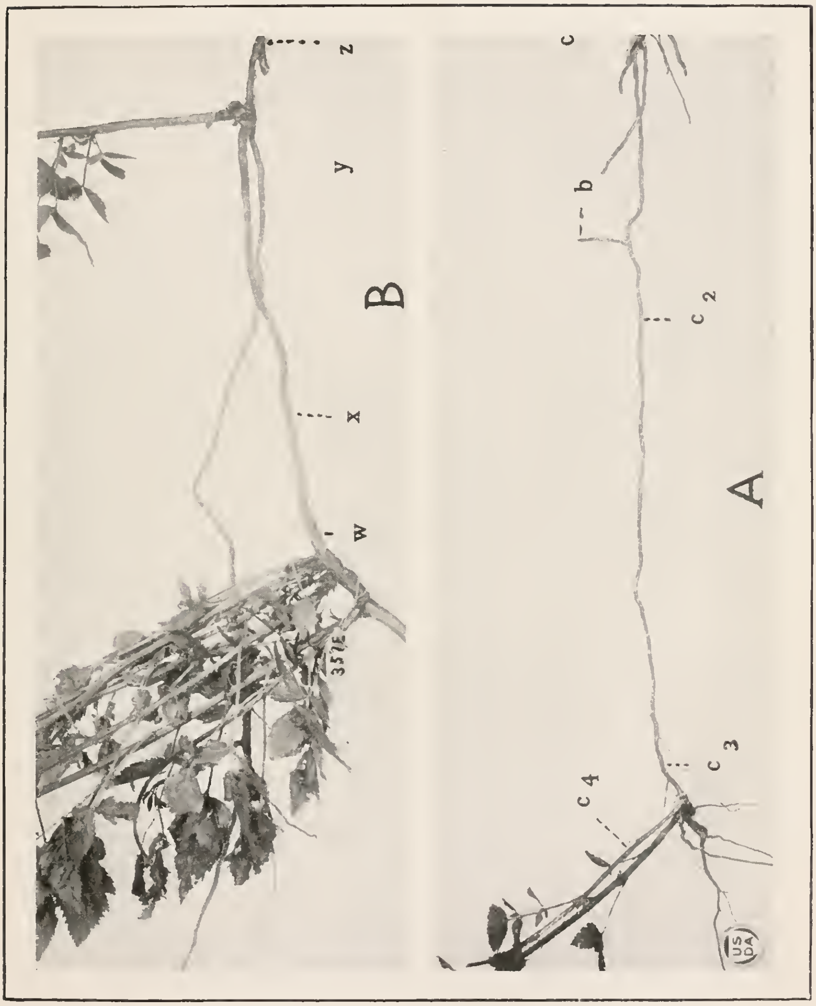
A.—Primary systemic infection of Kittatinny blackberry. The axial bud of the shoot inoculated in 1921 developed at once into a branch 2 feet long. The terminus of this branch, instead of being winter-killed, as always occurs in normal canes in our climate, remained alive and its terminal bud (a′) proliferated into a new shoot (a) in 1922. Mycelium was found only in the pith at a in the new shoot and at c in the old cane; at the node between the old and the new growth and where leaves were attached, mycelium was found in the pith and in the medullary ray gaps. A cross section of this cane at c is shown in Plate 2, C; second ring of wood, y, is being developed on the side below a proliferating shoot from an axial bud.

B.—Contrast the type of infection shown here with the localized infection shown in Plate 3. The old cane, originally inoculated when a shoot, had died during the early summer, but the mycelium was soon able to reach the root which lay near the surface of the soil, with the result that several buds were developed along the fusiform enlargement of the root, all of the new shoots being systemically infected. Since the fungus is now established in the runner, the root system must be destroyed to prevent the spread of the parasite. In case of a localized infection at the nodes some distance above the surface of the soil, the rust disappears along with the natural death of the cane at the close of the season.

PLATE 7

A.—Primary root infection of Mercereau blackberry No. 352 C. The parent plant giving rise to the horizontal root now showed a large healthy cane in blossom. A root 18 inches long and one-half inch in diameter branched freely at 12 inches, then gave rise to a slender runner; at b, 14 inches from a, a stub end of a 1921 cane cut off accidentally; 32 inches farther, two new shoots, infected, from a swollen region of the runner. Sections of the root at c, c_2, and c_3 showed no mycelium. Sections at c_4 of the new shoot showed hyphae in the pith only. This is clearly a case of root infection, although no wound scar is visible.

B.—Root attached to No. 357 E, a Kittatinny blackberry showing a case of primary root infection. The parent plant, at the right, was normal, free from rust, and bearing blossoms. Wound callus or scar on the root at w shows where the parasite entered the cortex of the root which lay very near the surface of the ground. No mycelium was found in the horizontal root at x, y, and z. See text for further discussion.


B
w
x
y
z
A
b
c
c 2
c 3
c 4

RESISTANCE IN RYE TO LEAF RUST, PUCCINIA DISPERSA ERIKSS.[1]

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INTRODUCTION

The leaf rust of rye, *Puccinia dispersa* Erikss., and its host are coextensive, for this disease has been found practically in all parts of the world where rye is grown. The literature on this rust refers to it under several different names. It belongs to that group of leaf rusts of cereals and wild grasses to which the name *P. rubigo-vera* (D C.) Wint. was for a long time applied. Eriksson and Henning (*9*)[3] separated this species into two, *P. glumarum* (Schm.) Erikss. & Henn., the stripe rust, and *P. dispersa* Erikss., the brown rust of cereals and wild grasses. On account of differences in hosts, a number of races were recognized within the brown rust by Eriksson (*6*), that on rye being designated as *P. dispersa* f. sp. *secalis.* Later the races on the other cereals and the wild grasses were raised to specific rank by Eriksson (*7*) and given binomial names, leaving the name *P. dispersa* for the rust found on rye. As such it has been most widely known in the literature which deals with this fungus as the cause of a disease of rye. Still other names, however, have been applied to it in mycological literature, such as *P. secalina* Grove, *P. asperifolii* (Pers.) Wettst., and *Dicaeoma asperifolii* (Pers.) Kuntze. A full list of such names is given by Arthur and Fromme (*1*) in the North American Flora.

The biology of this rust has been more or less completely worked out. *Puccinia dispersa* was shown by DeBary (*2*) to produce its aecia on *Anchusa officinalis* and *A. arvensis*, results which were duplicated by a number of others both in Europe and in this country. Apparently, however, this aecial stage usually is not necessary for the survival of the rust from year to year, since it has been observed to live over winter in the rye plant itself by Baudyš (*3*), Treboux (*13*), and others in Europe and by Carleton (*4*), Christman (*5*), and others in this country. That the other cereals and the wild grasses play no part in the overwintering and spread of this disease of rye is evident from the work of Eriksson (*6, 7*) and others, who have found that this rust is closely restricted to rye.

The severity of the disease varies in different regions, according to climatic conditions. Its severity also varies from year to year in any one locality as weather conditions vary, but it is always present to some extent. Under favorable conditions, such as years with mild winters and

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[2] The writers wish to acknowledge the efficient assistance of Mr. Leroy E. Compton, Junior Pathologist, Office of Cereal Investigations, in the laborious task of inoculating seedlings.

[3] Reference is made by number (italic) to "Literature cited," p. 251–252.

early springs with cool nights and heavy dews, the rust develops to a conspicuous extent in the principal rye-growing districts, so that by heading time the plants have a reddish appearance from the development of the uredinia of the rust. The rye plant, however, is not killed by the disease, and shriveling of the kernels by this rust has never been noted. Loss in yield is difficult to estimate, since the general prevalence of the rust does not permit any basis for comparison. On the other hand, it is hardly probable that heavy infections do not cause loss, for such infections destroy much photosynthetic tissue, draw heavily on the plant's supply of food material in the development of the rust and especially in its large spore production, and increase evaporation through the rupturing of the epidermis of the rye leaves. All these are factors which vary with the amount of infection, the vigor of the host plant, the condition of the soil, and the temperature and humidity of the atmosphere. As a result, opinions vary as to the amount of damage which may be produced. That the aggregate loss, however, may be considerable, is shown by the estimate made by the Plant Disease Survey of the United States Department of Agriculture [4] for the year 1919. This is based upon reports from the various pathologists throughout the United States, and, therefore, should be a fairly accurate average. According to this estimate, the loss due to the leaf rust of rye in the United States for 1919 was placed at 538,000 bushels, a third of the estimated reduction of yield of rye from all diseases in that year.

As with other rusts of the small grains, there is no feasible method of controlling leaf rust of rye by fungicides. Because of rather general winter survival of the rust in this country, elimination of the alternate host would be of little benefit, even if the latter occurred to any extent. Consequently, the discovery or development of a resistant strain of rye apparently offers the only promise of control of this disease. While the investigations of a number of workers have determined the susceptibility of rye as a species to specialized races of *Puccinia graminis* Pers. and *Erysiphe graminis* D C., as found on the other cereals and grasses, apparently no study has been made to determine whether varietal or individual differences exist in rye as to suceptibility to diseases which are specific to it. A few general field observations have been recorded. Sorauer (*11*) lists eight rye varieties as susceptible to "rust" in Germany and nine varieties as resistant. Vavilov (*15*) states that opinions vary as to the resistance of rye to *P. dispersa*, but that Jaczewski holds Champagne and the ordinary "bushy" variety to be resistant and Noviko notes resistance to leaf rust in Zealand, Danish Kampin, Probst, and Petkus rye.[5] Eriksson (*8*), in the case of the snow-mold disease, states that Petkus rye is resistant, while Zealand is susceptible.

As Vavilov (*15*) and others have pointed out, rye is a cross-pollinated plant with no sharply defined botanical varieties, the commercial varieties differing in being constituted of somewhat different complexes. Under such conditions, sharp varietal differences as to rust resistance are hardly to be expected, and the detection of resistant strains in such complexes is difficult, especially under field conditions, where the plants are intermingled so that individuals are not easily distinguished. The

[4] U. S. Department of Agriculture, Bureau of Plant Industry. Plant Disease Survey. Crop losses from plant diseases in the united states in 1919. In U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Bul. Sup. 12, p. 307-332. 1920.

[5] The writers wish to acknowledge the kindness of Mr. M. N. Levine, Assistant Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, in translating from the Russian Vavilov's statements concerning rust resistance in rye.

results reported in this paper were obtained through a study of rye seedlings in pot culture in the greenhouse, where individual differences are more easily distinguishable. The employment of this method has resulted in bringing out differences in resistance and gives promise of being of considerable importance in the improvement of rye through the development of disease-resistant strains.

DISCOVERY OF RYE RESISTANT TO LEAF RUST

In the fall of 1920, three kernels from a rye head, supposedly fertilized by wheat pollen in the experimental nurseries at Washington, D. C., were sown in the greenhouse of the Department of Botany, Purdue University Agricultural Experiment Station, at La Fayette, Ind. The resulting plants were there inoculated with the leaf rust of wheat, *Puccinia triticina* Erikss., and the leaf rust of rye, *P. dispersa*. Plants grown from seed from an open-fertilized head of the parent rye plant and from the variety of wheat from which pollen had been used in pollinating the rye head were used as controls. The inoculation with the leaf rust of wheat produced heavy infection on the wheat control but only slight infection on the three supposed hybrids and on the rye control. Inoculation with *P. dispersa*, on the other hand, produced no infection on the wheat control, while on the rye control, one plant, No. 10, was highly susceptible (Pl. 1, D) and another, No. 11, showed some resistance (Pl. 1, E), as indicated by the hypersensitive areas surrounding the uredinia. The three supposed hybrids showed an even greater variation, one plant, No. 7, being as susceptible (Pl. 1, C) as the susceptible control; the second, No. 9, being highly resistant (Pl. 1, A), as shown by the definite hypersensitive areas accompanying the uredinia; while the third, No. 8, was practically immune (Pl. 1, B) showing only a few small hypersensitive areas. Thirty-three other plants from open-fertilized seed of the parent rye plant were then inoculated with the leaf rust of rye, 19 showing a high susceptibility similar to that of plants Nos. 7 and 10, 5 showing some slight signs of resistance, 5 showing a resistance similar to Nos. 9 and 11, and 4 a high resistance similar to No. 8. The similarity in action of the supposed hybrids and the plants grown from open-fertilized rye, at once threw doubt upon their hybrid nature. When grown to maturity all proved to be pure rye, as was indicated by their reaction to the rust.

SECOND GENERATION RESULTS FROM RESISTANT PLANTS

The first generation of the progeny from plants 7, 8, 9, and 10 has been studied as to susceptibility to leaf rust, in an attempt to throw some light on the nature and inheritance of resistance, with the ultimate object of obtaining pure rust-resistant strains of rye. In the spring of 1921, plants 7, 8, 9, and 10 were selfed by bagging the heads of each plant separately, and were crossed in various combinations by bagging the heads of two plants together in combinations as shown in Table I. From the seed obtained from these plants, selfed, crossed, and open-fertilized, 596 plants were raised in the greenhouse at La Fayette, Ind., in the fall of 1921. Each was grown in a 3-inch pot, and when in about the third or fourth leaf, was tested as to its susceptibility to leaf rust. After being studied as to their susceptibility, a select few of each of the principal types were transplanted to 10-inch pots and were selfed and crossed in various combinations again in the spring of 1922. The re-

maining plants were all transplanted to the field, where they also were selfed and crossed in the spring of 1922, thereby furnishing considerable material for further study.

TABLE I.—*Number of seeds of rye obtained by selfing and by crossing plants 7, 8, 9, and 10 in various combinations*

Plant No.	Treatment.	Number of heads.[a]	Number of kernels produced.
7	Selfed	2	8
8	.....do	2	0
9	.....do	2	0
10	.....do	5	3
7	Crossed by 8	1	9
7	Crossed by 9	2	6
7	Crossed by 10	1	17
8	Crossed by 7	1	15
8	Crossed by 9	5	105
9	Crossed by 7	2	16
9	Crossed by 8	5	46
10	Crossed by 7	1	4
	Total	....	229

[a] The remaining heads in all cases were open-fertilized and produced a total of 367 seeds.

Material differences were found in types of susceptibility in the specimens studied. Nearly all kinds of intergradation between extreme susceptibility and practically complete immunity were noted. These, however, may be divided into about nine main types as shown in Plate 2, A–I.[6] Thus one group showed a high susceptibility (Pl. 2, A) as indicated by the very large size and dark color of the uredinia, approaching in appearance those of *Puccinia graminis*. Under greenhouse conditions this type usually produced from the outer portion of the mycelium a more or less perfect ring of uredinia encircling the one or two first formed. Another group (Pl. 2, B) showed a somewhat less vigorous development, the uredinia being smaller, somewhat lighter in color, and the encircling uredinia being produced less frequently. A few individuals, having a type of susceptibility very similar to the last, showed also a few small uredinia in hypersensitive areas (Pl. 2, D). This condition may possibly indicate the presence of more than one strain of the rust in the culture used or may represent a distinct type. Another group (Pl. 2, E) had uredinia of fair size similar to the preceding, differing in that, while the host tissue in the infected areas did not show any especially deleterious effect, the tissue immediately surrounding these areas became chlorotic and in some cases brown, resulting in the infected areas appearing as green islands. Another group (Pl. 2, C), although having fairly large uredinia, showed a lack of normal adjustment between host and rust in the more or less mottled or chlorotic condition of the host in the infected areas. All of the remainder showed pronounced resistance as indicated by hypersensitive areas which developed in the infected spots. There

[6] Plant 8 (Pl. 1, B) is of the same type of susceptibility as that shown in Plate 2, I; plant 9 (Pl. 1, A) as that in Plate 2, H; plant 11 (Pl. 1, E) as that in Plate 2, F; plants 7 and 10 (Pl. 1, C, D,) as those in Plate 2, A or B. Leaves of the parent plants 7, 8, 9, and 10 were photographed natural size, shortly after infection had appeared, while the types given in Plate 2 were photographed, enlarged two diameters, after the rust had reached its fullest development. This accounts for the single scattered uredinia shown on the susceptible parents and the encircling uredinia in type A, Plate 2.

were various degrees of resistance among these, some having uredinia of normal size each surrounded by a large, sharply defined killed area (Pl. 2, F); others having uredinia much reduced in size but usually accompanying each hypersensitive area (Pl. 2, G); still others in which the hypersensitive areas were numerous and definite but only occasionally containing a small uredinium (Pl. 2, H); and finally those where the only sign of infection was a few more or less indefinite hypersensitive areas, no uredinia being produced (Pl. 2, I). In all cases where hypersensitive areas were present, they were of fairly good size, nothing which might be called flecking apparently being produced.

Vavilov (*14*) gives a system of classification for the types of susceptibility shown by wheat varieties to the leaf rust of wheat, *Puccinia triticina*, a rust very similar in many ways to the leaf rust of rye. He states that this system is a modification of that used by Eriksson, in which five degrees are recognized and are designated by numerals, from 0 (no pustules) to 4 (very pronounced susceptibility). Vavilov, besides using the number of pustules produced, considers the character of development of the fungus of importance, such as the presence of killed areas with or without uredinia. A somewhat similar system of classification has been used by Stakman and Levine (*12*) for the susceptibility of wheat varieties to stem rust, *P. graminis*. In a similar system of classification, the types of leaf rust infection of rye should be arranged as follows:

0. No uredinia formed; hypersensitive areas sometimes present and definite, sometimes faint or absent. Plate 2, I.

1. Uredinia few, minute, in the center of definite hypersensitive areas; few to many hypersensitive areas without uredinia. Plate 2, H, G.

2. Uredinia fairly abundant, moderate in size but always surrounded by hypersensitive areas; hypersensitive areas seldom without uredinia. Plate 2, F.

3. Uredinia abundant, moderate in size, without hypersensitive areas but in some cases surrounded by slightly chlorotic tissue. Plate 2, C, B.

4. Uredinia abundant, very large, hypersensitiveness absent but uredinia occasionally in green islands. Plate 2, A, E.

X. A combination of several of the above types appearing on the same leaf, some uredinia large and without hypersensitiveness, others small and accompanied by hypersensitive areas.[7] Plate 2, D.

The manner of inheritance of rust resistance can not be determined from the results thus far secured. The results obtained from the crosses between the two resistant plants 8 and 9, however, are of interest at this time. From the seed obtained as the result of these crosses, 111 plants were grown. Of these, 2 showed a high susceptibility like A, Plate II; 7 were like B; 9 like C; 11 like D; 19 like F; 38 like G; 17 like H; and 8 like I. In other words, two rye plants showing high resistance, when crossed may produce in their offspring almost all degrees of susceptibility. As shown in Table I, the other crosses and the selfs furnished only 78 kernels altogether. The plants grown therefrom did not furnish additional evidence from which definite conclusions could be drawn. Studies of the inheritance of susceptibility and resistance are being continued with this material.

[7] In some cases this mixture of types of infection may indicate a mixture of strains of the rust; but reinoculations in a few cases with the large uredinia from such mixed types have continued to give the mixed type. These cases, in consequence, would fall into the heterogeneous class X established by Stakman and Levine (*12*).

RESISTANCE OF RYE VARIETIES TO LEAF RUST

Further investigations concerning the susceptibility of rye to leaf rust were carried on in the autumn of 1921 to determine the susceptibility of a number of the principal rye varieties. Fifty-nine selections grown at Arlington Experiment Farm, near Washington, D. C., were obtained. All of these had been grown for two or more years in adjacent rows and, consequently, some crossing probably had taken place. That there still existed considerable individuality in them, however, was shown by the variations in yield observed in the 1921 harvest. Six varieties of winter rye and three of spring rye were obtained from Mr. R. R. Mulvey of the Soils and Crops Department, Purdue University Agricultural Experiment Station. Of these, the Rosen and Wisconsin No 2 varieties had just been obtained from the Michigan and Wisconsin agricultural experiment stations, respectively, where precautions are taken to maintain their purity. The other varieties had been grown in close proximity for several years and doubtless had crossed. Additional pedigreed seed of Rosen rye was obtained from Prof. J. F. Cox, of Michigan Agricultural College, where this variety is maintained in a pure condition. Seed of Abruzzes rye was obtained from Prof. G. M. Garren, of the North Carolina Agricultural Experiment Station, where it is the leading variety and, therefore, probably quite pure.

TABLE II.—*Data on resistance of 70 varieties and selections of rye to leaf rust, Puccinia dispersa, at La Fayette, Ind., in 1922*

Variety.	C. I. No.[a]	Source.	Number of plants inoculated.	Resistant plants.	
				Number.	*Per cent.*
Abruzzes		N. C. Agr. Exp. Sta	81	7	8. 6
Do		Ind. Agr. Exp. Sta	38	3	7. 9
Do	40- 1	Cereal Inv	92	4	4. 3
Do	40- 2	do	72	5	6. 9
Do	40- 3	do	84	5	5. 9
Do	40- 4	do	83	5	6. 0
Do	40- 5	do	80	3	3. 7
Do	40- 6	do	72	3	4. 2
Do	40- 7	do	88	5	5. 7
Do	40- 8	do	63	1	1. 6
Do	[b] 40-47	do	87	4	4. 6
Do	[b] 40-48	do	71	7	9. 9
Do	[b] 40-49	do	73	3	4. 1
Do	[b] 40-55	do	35	7	20. 0
Do	[b] 40-56	do	29	3	10. 3
Do	[b] 40-57	do	29	6	20. 7
Do	[b] 40-59	do	48	7	14. 6
Do	[b] 40-61	do	63	4	6. 3
Common Spring		Ind. Agr. Exp. Sta	134	9	6. 7
Giant Winter	30- 9	Cereal Inv	57	1	1. 8
Do	30-11	do	61	2	3. 3
Do	30-12	do	57	1	1. 8
Do	30-13	do	91	1	1. 1
Do	30-14	do	59	2	3. 4
Do	30-15	do	56	3	5. 4
Do	30-16	do	38	1	2. 6
Do	30-17	do	48	1	2. 1

[a] Numbers preceding dash are Office of Cereal Investigations accession numbers; those following dash are row number in the Arlington Experiment Farm nursery at Washington, D. C., in 1921, and represent selections (made in 1918) or strains, in most cases.
[b] Selection made previous to 1918.

TABLE II.—*Data on resistance of 70 varieties and selections of rye to leaf rust, Puccinia dispersa, at La Fayette, Ind., in 1922*—Continued

Variety.	C. I. No.	Source.	Number of plants inoculated.	Resistant plants.	
				Number.	*Per cent.*
Giant Winter	30–18	Cereal Inv	34	1	2.9
Do	30–19	....do	44	3	6.8
Do	30–21	....do	50	1	2.0
Do	30–22	....do	43	1	2.3
Do	30–23	....do	42	1	2.4
Henry	138–25	....do	40	1	2.5
Do	138–26	....do	47	1	2.1
Do	138–27	....do	59	5	8.5
Do	138–28	....do	73	1	1.4
Invincible	207–46	....do	92	5	5.4
Ivanov	152–29	....do	66	1	1.5
Do	152–31	....do	64	5	7.8
Do	152–32	....do	49	2	4.1
Mammoth Winter		Ind. Agr. Exp. Sta	63	4	6.3
Mexican	[b] 108–62	Cereal Inv	51	5	9.8
Petkus		Ind. Agr. Exp. Sta	63	7	11.1
Prolific Spring		....do	151	10	6.6
Rosen		....do	59	11	18.6
Do		Mich. Agr. Exp. Sta	89	16	18.0
Do	[c] 195–45	Cereal Inv	92	10	10.9
St. John	130–33	....do	44	1	2.3
Do	130–43	....do	78	4	5.1
Do	[b] 130–63	....do	56	7	12.5
Select Spring		Ind. Agr. Exp. Sta	169	7	4.1
Star		....do	76	16	21.0
Virginia	128–24	Cereal Inv	44	1	2.3
Von Ruemker No. 1	173–37	....do	65	4	6.2
Do	173–44	....do	82	1	1.2
Do	[b] 134–52	....do	76	7	9.2
Do	[b] 134–53	....do	108	5	4.6
Von Ruemker No. 2	174–38	....do	60	2	3.3
Do	174–42	....do	78	4	5.1
Wisconsin No. 2 (Schlanstedt).		Ind. Agr. Exp. Sta	77	4	5.2
Unnamed	34	Cereal Inv	56	6	10.7
Do	[d] 39	....do	70	6	8.6
Do	[e] 41	....do	77	5	6.5
Do	[f] 132–51	....do	85	16	18.8
Do	[b] 54	....do	33	2	6.1
Do	[b] 58	....do	54	8	14.8
Do	[c] 178–64	....do	52	8	15.4
Do	[c] 183–65	....do	44	1	2.3

[b] Selection made previous to 1918.
[c] Not selections; 178–64 from Taurida, Russia, S. P. I. No. 38692; 183–65 an unnamed lot of seed from Utah.
[d] Selection made at Cobleskill, N. Y., 1918.
[e] Selection made in Tennessee, 1918.
[f] Selection made at Cornell University, Ithaca, N. Y., in 1912.

These varieties and strains were tested by sowing a number of pots of each, about 10 kernels being sown to a pot. When the seedlings were in about the second or third leaf, they were inoculated with a culture of leaf rust obtained from volunteer rye at La Fayette, Ind., and maintained in the greenhouse as stock material. Infection appeared in from 7 to 10 days and when the rust had reached its maximum development, usually about 2 weeks after inoculation, notes were taken. Table II

shows the results obtained. Plants showing types of infection 0, 1, and 2 are listed as resistant. A few of the more highly resistant plants of a number of the varieties and strains were transplanted to larger pots and grown to maturity, being crossed and selfed to obtain material for further work.

An examination of Table II shows that one or more resistant plants were found in each of the varieties and selections. Considerable variation apparently exists as to the number of resistant individuals to be found in these varieties and selections. This may be due in some cases to the relatively small number of plants which it was possible to study. The differences between such varieties and selections as Rosen, Abruzzes 40–55, 40–56, 40–59, 40–48, and Star on the one hand, and Giant Winter, Henry, and Virginia on the other, probably represent varietal differences in the occurrence of resistant strains. It may be that a number of the strains of the latter varieties, when in the pure condition, are entirely susceptible, the small degree of resistance shown coming from cross fertilization with adjacent more highly resistant strains. It is, however, significant that such varieties as Rosen from Michigan, Abruzzes from North Carolina, and the spring ryes from Purdue University, all fairly pure varieties, show resistance. The data indicate that to some extent resistance is to be expected in all the varieties of rye now commonly grown.

DISCUSSION

The discovery of rye individuals resistant to leaf rust of rye is of considerable interest because of the lack of data or observations on disease resistance in this cereal. Although 68 selections of rye representing about 17 of the principal varieties grown in this country were studied, no variety was found which was uniformly resistant. As rye is almost always cross-pollinated, this would be expected unless a variety was selected with rust resistance in view. On the other hand, all the selections studied showed at least a few resistant individuals. This indicates, at least in all the varieties studied and probably in others as well, that the factor or factors determining resistance have not been eliminated by the processes which selected varieties from the original parental stock. Rye varieties have been obtained largely by repeated selection of desirable types without precautions being taken to prevent cross pollination. As a result, the varieties are relatively few and ill-defined, differing mostly in ability to develop and yield well, and composed of many strains, at least so far as disease resistance is concerned. The differences shown between the various selections and varieties as to proportion of resistant individuals may be due to a difference in the number of the susceptible and resistant strains of which they may be considered to be constituted. The constant crossing and recrossing which must occur among these strains doubtless cause the number of resistant individuals to vary considerably, so that any one test is probably only a rough estimate. Before the exact degree of resistance in the varieties can be determined, it doubtless will be necessary to establish, if possible, a number of pure lines by repeated selfing and selection similar to the methods being employed with corn in this country.

The data obtained are insufficient to justify drawing conclusions as to inheritance in rye of resistance to leaf rust. It is obvious that a number of generations of breeding will be necessary before the genetic constitution of material of such complexity can be known with any degree

of accuracy. The results obtained by crossing the two plants, 8 and 9, resistant to leaf rust, however, are suggestive. The appearance of two highly susceptible and seven susceptible plants in the progeny from this cross strongly indicates that resistance is dominant. The appearance of so many different types in the offspring is confusing. Whether more than one pair of factors is involved, or one main pair with modifying factors, as Puttick (*10*) has suggested as an explanation for the appearance of different types of susceptibility to *Puccinia graminis* in segregates from a Marquis-Mindum wheat cross, or whether we have a number of segregating strains of rye which in homozygous condition may differ in respect to type of susceptibility, must be determined by future study. It is evident, however, that the problem of obtaining rust-resistant strains of rye is complicated not only by the high degree of self sterility and the consequent slow progress which can be made by selfing, but also by the dominance, as seems probable, of the desired quality of resistance and the consequent longer process of breeding before it is certain that a pure homozygous strain has been obtained.

SUMMARY

(1) Rye plants have been found which show high resistance to and in some cases practically complete immunity from the leaf rust of rye, *Puccinia dispersa* Erikss.

(2) Sixty-eight selections and varieties of rye, including such varieties as Abruzzes, Giant Winter, Henry, Invincible, Ivanov, Mammoth Winter, Mexican, Petkus, Rosen, St. John, Star, Von Ruemker, Wisconsin No. 2 (Schlanstedt selection), and a number of unnamed introductions, were studied as to susceptibility to leaf rust.

(3) None of these varieties or selections was uniformly resistant.

(4) All of these varieties or selections showed at least a few individuals having high resistance.

(5) Crosses made by bagging heads of two highly resistant plants together showed gradation in the susceptibility of the plants produced, varying from high susceptibility through intermediate grades of resistance to complete immunity.

(6) The production of susceptible individuals from a cross between resistant ones indicates that resistance probably is dominant. The production of intermediate types, however, would indicate complicating factors.

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PLATE 1

Types of susceptibility shown by rye plants to leaf rust, *Puccinia dispersa*. All natural size.

A.—Leaf showing the resistance of rye plant 9. Note the hypersensitive areas and the small uredinia.

B.—Leaf showing the high resistance of rye plant 8. Note the few hypersensitive areas and lack of uredinia.

C.—Leaf showing the susceptibility of rye plant 7. Note the large uredinia and lack of hypersensitiveness.

D.—Leaf showing the susceptibility of rye plant 10. Note the large uredinia and lack of hypersensitiveness.

E.—Leaf showing the moderate resistance of rye plant 11. Note the hypersensitive areas and the large uredinia accompanying each.

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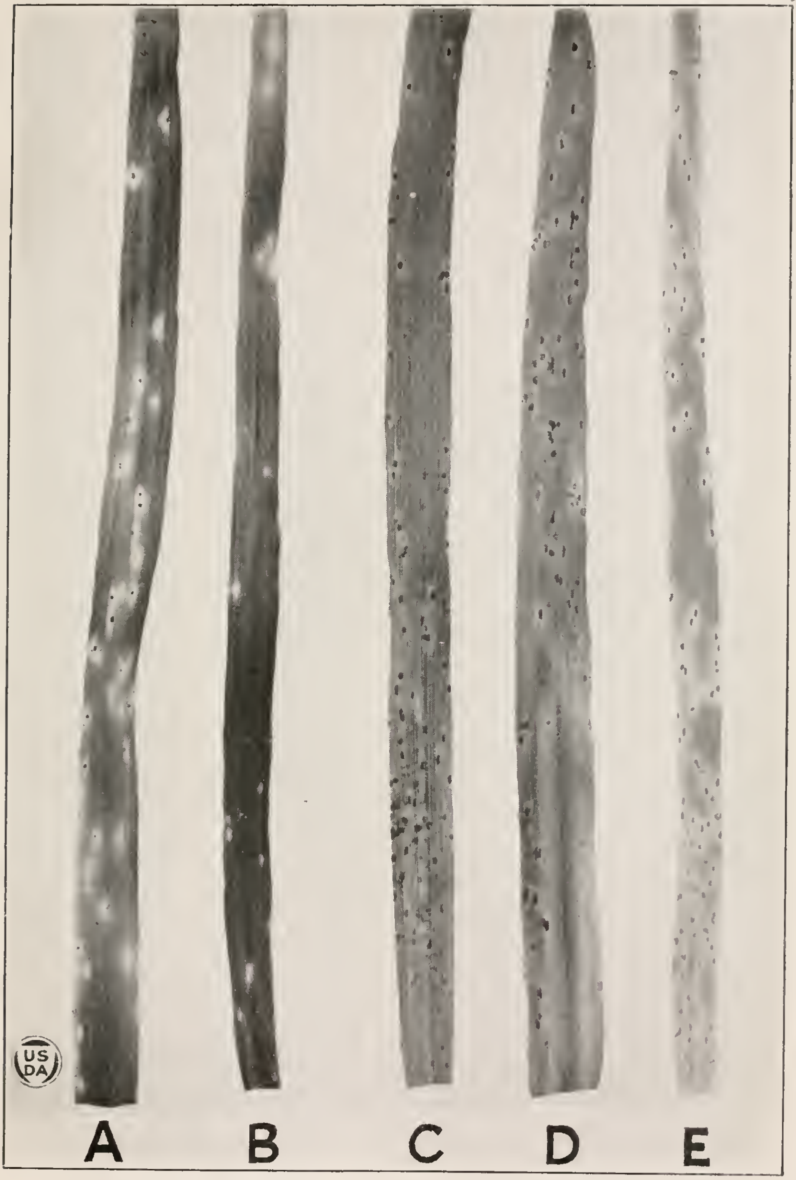

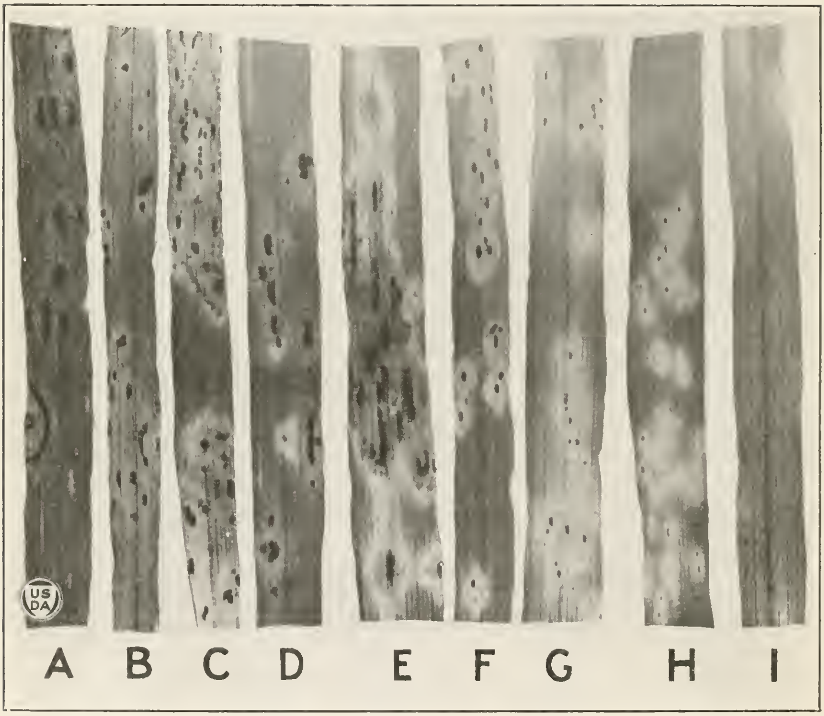
A B C D E F G H I

PLATE 2

Types of susceptibility shown by offspring of selfs and crosses between rye plants 7, 8, 9, and 10 to leaf rust of rye, *Puccinia dispersa*. X2.

A.—Uredinia very large, often circular. Type 4.

B.—Uredinia midsized, less often circular. Type 3.

C.—Uredinia midsized, infected areas somewhat chlorotic. Type 3.

D.—Uredinia midsized or large, sometimes accompanied by definite hypersensitive spots. Type X.

E.—Uredinia midsized or large, infected areas green, bordered by chlorotic tissue. Type 4.

F.—Uredinia midsized in large, definite hypersensitive spots. Type 2.

G.—Uredinia small in less definite hypersensitive spots, the latter sometimes without uredinia. Type 1.

H.—Hypersensitive areas abundant, only occasionally containing small uredinia. Type 1.

I.—Hypersensitive areas few, indefinite, no uredinia produced. Type 0.

AN UNDESCRIBED ORANGE PEST FROM HONDURAS[1]

By A. C. Baker

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When specimens of the "citrus blackfly" (*Aleurocanthus woglumi* Ashby) were first studied by Dr. A. L. Quaintance and the writer, there was no indication that in a few years it would become one of the most important of orange pests. It was known only as an undescribed aleyrodid occurring on the orange in certain parts of India and Ceylon. Apparently it was held in check there by natural factors. Its new environment in the Western Hemisphere, however, has lacked these factors.

In March, 1920, Dr. W. M. Mann took a species of this same family (Aleyrodidae) at Ceiba, Honduras, where it occurred abundantly on orange. As will be seen from Plate 2, the species is controlled in its natural environment by the attacks of parasites, and it is therefore of potential importance to the citrus-growing regions of other countries where it might become established without these parasites. It seems wise to present a description of it, so that those interested may be acquainted with its appearance.

Aleurodicus (Metaleurodicus) manni, n. sp.

Egg.—Length 0.256 mm., width 0.096 mm. Color yellowish, sometimes with a brownish cast. Shape regular, not conspicuously flattened; stalk at extremity of egg, short. The stage available for study has a spherical orange colored body in the center of the egg.

First instar (larva) (Pl. 1, A).—Length 0.32 mm., width 0.14 mm. Color under the microscope transparent, with the exception of some orange-yellow on the abdomen and the purple eye spots. Margin entire. Twelve pairs of spines present, five pairs on the thorax and seven pairs on the abdomen, caudal and caudo-lateral pairs longest. Compound abdominal wax pores not visible. Vasiform orifice subcordate with the lingula included and rather broad distad. Abdominal segments distinct. Antennæ (Pl. 1, B) of three segments, long, slender, without bend, and tipped with a stout hair. Legs (Pl. 1, C) extending considerably beyond the margins of the body, distal segment with one rather long curved claw and a stout spine, proximal segment with a long hair.

Second instar (larva) (Pl. 1, D).—Length 0.48 mm., width 0.304 mm. Color similar to that of the first instar. Margin entire. Vasiform orifice subcordate, with the lingula large but shaped similarly to that found in the pupa case. On the caudal portion of the dorsum, in the region occupied by the caudal pair of pores in later instars, is a pair of small porelike structures in which a central process can be distinguished (Pl. 1, E); on the thorax also are present two pairs of about equal size, one pair just cephalad of the eyes and the other pair half way between them and the abdominal line. Legs short and thick, terminating in a long spine. Within margin is a series of small setae.

Third instar (larva) (Pl. 1, F).—Length 0.656 mm., width 0.496 mm. Color pale yellowish or brownish, the yellow prominent on the abdomen. Small eye spots purple. Margin entire. Vasiform orifice (Pl. 1, G) subcordate, lingula large and armed with two pairs of spines. Thorax with two pairs of reduced compound pores but these situated apparently in a different region from those found in the previous instar. Pores (Pl. 1, H) with a distinct central process. Twelve pairs of spines present on the submarginal area Legs heavier and shorter than in the previous instar.

[1] Accepted for publication May 2, 1923.

Fourth instar (pupa case) (Pl. 1, I).—On the leaf the pupa case (Pl. 2) appears entirely covered with a mealy white secretion and rests upon a short wall of compact wax which remains as a ring when the case is removed. From the largest pair of abdominal compound wax pores, the most anterior pair, two large, rather coarse wax rods extend for long distances in graceful curves. The two pairs caudad of this largest pair possess similar wax rods, but these rods are more slender and somewhat shorter. The more caudal pairs of pores are without rods. There appears to be no woolly secretion present. A pair of rods also extends from the cephalic pair of pores. Under the microscope pale yellowish brown in color or almost entirely transparent whitish. Size about 0.992 by 0.72 mm. Compound pores of seven pairs, of which one pair is cephalic and six pairs abdominal; the three caudal pairs, near the vasiform orifice, are greatly and equally reduced, being similar to the two reduced caudal pairs commonly found in species of Aleurodicus (Pl. 1, J); the two pairs cephalad of these only partially reduced, being functional as indicated by the wax rods extending from them (Pl. 1, K); the anterior abdominal pair largest and most complete and situated slightly farther mesad than the other functioning pores (Pl. 1, L); cephalic pores quite similar in general appearance to the median abdominal ones. Margin entire. Dorsum without any noteworthy sculpture. Vasiform orifice somewhat cordate but with the anterior margin straight; operculum filling less than half of the orifice; lingula extending to the caudal margin of the orifice, occasionally a trace beyond, armed with four stout, curved, spinelike hairs. The presence of the compound pores in the pupa case as compared with the earlier instars is worthy of note. Certain of those present in the second instar seem to be lacking in the third and reappear again in the pupa.

Fifth instar (adult female).—The adults available for study were preserved dry and therefore are not in a good condition to study, the more delicate parts being greatly shrivelled or broken. Length from vertex to tip of ovipositor 0.096 mm. Color brownish yellow with a greenish tinge. Wings transparent, except for the powdery covering and a slight clouding, under the microscope clear transparent, with the costal margins reddish brown. Length of forewing 1.36 mm., width at the junction of R_1 and R_s 0.592 mm., greatest width 0.64 mm. Antennæ not in a condition for study, but evidently rather long and slender and imbricated. Vasiform orifice prominent with a slender lingula.

Fifth instar (adult male).—Unknown.

Type.—Cat. No. 26072 United States National Museum.

Described from eggs, larvæ, pupæ, and adult females in balsam mounts, and pupa cases, etc., dry upon the foliage.

PLATE 1

Aleurodicus (Metaleurodicus) manni

A.—First instar (larva).
B.—Antenna of first instar.
C.—Leg of first instar.
D.—Second instar (larva).
E.—Porelike structure and central process on caudal portion of dorsum.
F.—Third instar (larva).
G.—Vasiform orifice of third instar.
H.—Pore and central process of third instar.
I.—Fourth instar (pupa case).
J.—Compound pore of a caudal pair of fourth instar.
K.—Compound pore of fourth instar with wax rods extending from it.
L.—Compound pore of anterior abdominal pair of fourth instar.

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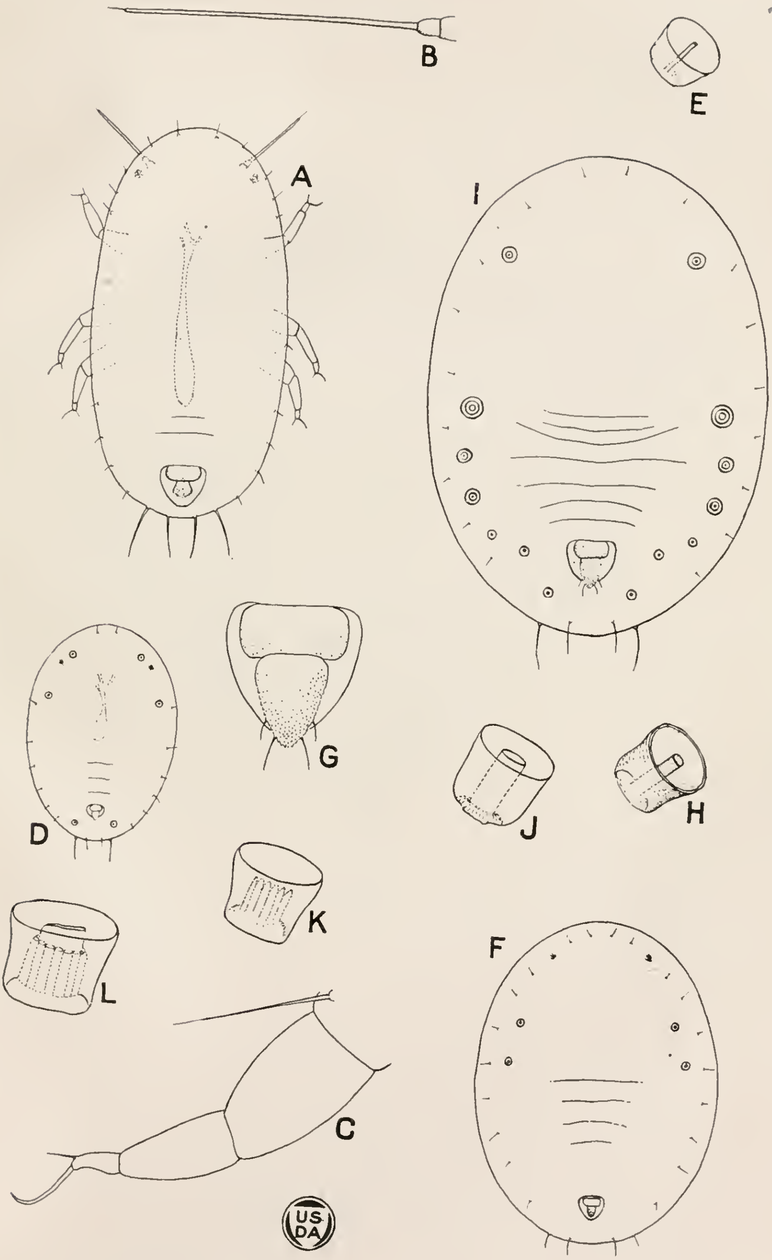

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PLATE 2

Aleurodicus (Metaleurodicus) manni

Infested orange leaf showing exit holes of parasites in the pupa cases.

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CORRELATION OF FOLIAGE DEGENERATION DISEASES OF THE IRISH POTATO WITH VARIATIONS OF THE TUBER AND SPROUT [1]

By Alfred H. Gilbert

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INTRODUCTION

It has been previously shown through the observations and experiments of a number of investigators that mosaic and leafroll of the Irish potato (*Solanum tuberosum*) are transmitted from season to season by means of the tubers. Among those who have contributed chiefly to this conclusion are, Orton (*9*),[2] Appel (*1*), Wortley (*15*), Stewart (*13*), Murphy (*8*), Melhus (*7*), Schultz et al. (*10*), and Schultz and Folsom (*12*). Since these diseases although carried in the tissues of the tubers do not cause any observable changes in their outward appearance, there would seem to be no means of distinguishing between healthy and diseased specimens when unsprouted tubers are held under favorable storage conditions. The observations and experiments reported in this paper were undertaken, therefore, with the purpose of discovering whether there were not some distinguishing characteristics associated either with the earlier or later stages of germination of tubers which might be utilized in determining the presence of mosaic or leafroll therein during the latter part of the storage period. It is evident that such correlations, if existent, would add to the knowledge of the diseases concerned by furnishing additional means of diagnosis and might also be a basis for the elimination of undesirable seed from stocks to be used for planting. The essential results of these investigations were reported in abstract at the Toronto meeting of the American Association for the Advancement of Science in December, 1921, and published in a subsequent number of Phytopathology (*6*).

Studies made at the Vermont Agricultural Experiment Station seem to indicate that certain definite correlations exist between both dormant and sprouted tubers and foliage condition in the plants grown from such tubers, and these studies were supplemented by observations both in the greenhouse and in the field as to variations in the seriousness of mosaic and leafroll symptoms in successive generations of plants and other related points.

In April, 1921, the noteworthy paper by Schultz and Folsom entitled "Leafroll, Net-Necrosis and Spindling-Sprout of the Irish Potato" (*12*) appeared, and though much wider in scope than the present paper and developed along different lines, yet certain of the most significant conclu-

[1] Accepted for publication May 2, 1923.
[2] Reference is made by number (italic) to "Literature cited," p. 265-266.

sions closely duplicate and corroborate those of the present investigation. This is notably true of the correlations shown between spindling-sprout and net-necrosis of the tuber and leafroll of the foliage. In addition to these correlations, the studies reported herein furnish evidence on other matters related to tuber germination and foliage symptoms—namely, (*a*) retardation in the sprouting of mosaic and leafroll tubers, (*b*) bud uniformity in mosaic and leafroll tubers, (*c*) the simultaneous occurrence of both mosaic and leafroll symptoms in the same plant, (*d*) variation in the progressive seriousness of mosaic infection from season to season, and (*e*) apical dominance in its relation to disease.

GERMINATION TESTS

During the winters of 1919–20 and 1920–21 many tubers of known history as to the condition of the plants from which they came were germinated. The germinations were made in the greenhouse under favorable conditions of moisture and temperature, the seed pieces being planted in soil in benches and pots or in sawdust and excelsior in wide shallow pots. In the course of the trials it was found that tubers from a crop planted in May and harvested in October did not germinate normally until the following January or February. In the length of the rest periods thus indicated the facts observed conform in general to the observations of Appleman (*2*), who found that the rest periods of Irish potatoes differed to some extent between varieties but were quite uniform within a certain variety. He found that while in general the rest period was terminated after about 90 days, it could be very much shortened by removing the skins from the tubers without injury to the buds. In the course of the investigations herein reported, earlier germination was secured by using Appleman's method, which, however, did not prove to be adapted to the needs of the present studies. The most satisfactory results were secured from germinations made toward the end of the storage season, during February, March, and April, while some of the most significant observations on spindling-sprout were made in the case of tubers sprouting in the storage cellar at the very end of the storage period. The cellar in which the tuber samples were kept during the winter was sufficiently moist so that the potatoes did not shrivel, and registered temperatures ranging from 35 to 45° F. throughout the winter months, rising with the higher temperatures of April and May. At planting time most of the tubers had developed sprouts of varying lengths.

RETARDATION IN SPROUTING

As a result of germinating many tubers both diseased and healthy in the greenhouse, evidence has accumulated that germination of the seed piece and growth of the young shoot are definitely and markedly retarded both in the case of serious mosaic and of serious leafroll infections. In instances of mild degrees of infection, however, little if any variation from the normal appearance in sprouting can be detected. In order to secure evidence on this point, tubers of the Green Mountain variety were used, some of them of known history as to the presence or absence of disease for one or more seasons, while others were derived from general stock. One half of each tuber was planted in a shallow pot on moist sawdust and covered with excelsior, and the other half was planted in soil in an 8-inch pot. All were given uniform treatment as to light, moisture, and temperature. The appearance and rate of

growth of the shoots from the diseased as compared with those from the healthy tubers were observably unlike. Slower growth occurred and a pinched, unhealthy and slightly abnormal appearance characterized the sprouts from tubers seriously affected with either mosaic or leafroll. Plate 1, A (two potted plants and sprouted seed pieces from the same corresponding tubers), illustrates this point. The plant at the left exhibits extreme mosaic symptoms and the sprouts of the seed piece distinct retardation in growth; the plant at the right, mild mosaic symptoms and a nearly normal sprout on the seed piece. Plate 1, B, shows another case of retarded sprouting, the potted plant being from the same tuber as the sprouted seed piece and exhibiting extreme mosaic symptoms. The plants shown in Plate 1, B, were from a parentage described as "healthy but adjacent to mosaic hills." Plate 1, A, shows an additional evidence of weakness and degeneration in the brown lesions on the several sprouts of the seed piece at the left. Schultz and Folsom (*11, p. 321*) have reported a "spotting and streaking of the leaves, petioles and stems" apparently somewhat similar to the foregoing, accompanying mosaic symptoms induced in potato plants by artificial inoculation.

RELATION OF MOSAIC AND LEAFROLL IN VERMONT

Schultz and Folsom (*12, p. 75*) note that in northeastern Maine the Irish Cobbler is the only variety which shows more than a trace of leafroll in commercial fields and that it "seldom shows more than a trace of mosaic, while the Green Mountain and Bliss Triumph varieties are seldom found with only a trace of mosaic."

In Vermont the facts with reference to these points are somewhat different. As in Maine, Irish Cobblers are more subject to leafroll than to mosaic, but this variety in Vermont frequently shows more than a "trace of mosaic." Further, Green Mountains in Vermont are now quite as commonly, though in general not as seriously, affected with leafroll as with mosaic. The State certified seed-potato inspectors during the past two or three seasons have found fields with relatively high percentages of mosaic much more frequently than fields with high percentages of leafroll. It is also a common experience now in this State to find in many stocks of Green Mountains, and less frequently in Irish Cobblers, both mosaic and leafroll infection in the same field. The percentages of mosaic found are frequently reported as "only a trace." In these respects the conditions in Vermont with reference to mosaic and leafroll occurrence are appreciably different from those in Maine.

SIMULTANEOUS OCCURRENCE OF MOSAIC AND LEAFROLL IN THE SAME PLANTS

Stewart (*13*) in 1916, in recording his observations on "some degenerate strains of potatoes," reports that "certain hills were much affected with leafroll and showed also traces of mosaic." In studying the symptoms of mosaic and leafroll plants both in the field and in the greenhouse, it has frequently been observed by the writer that a plant may exhibit the characteristics of both diseases at the same time. Instances of this have been noted both in the early part of the season and later. When the symptoms appeared very early it was obvious that the double infection had been carried in the seed tuber, while later appearances might have been due to secondary infections of one or both diseases. In most cases where this double infection was observed, the unhealthy

condition of the plant was very marked. There was a spindling habit, foliage area was reduced, and in some instances brown necrotic regions had developed on leaves, petioles, and stems, and premature defoliation had occurred. Plate 1, C, shows a plant in which these symptoms were observed under greenhouse conditions.

In Table I it will be noted that several samples are recorded as exhibiting both mosaic and leafroll symptoms. In these cases the leafroll may perhaps be considered as the more significant since it is here associated with net-necrosis and spindling-sprout. The two infections combined seem to produce a condition of extreme weakness and low yield, more marked than would result, except occasionally, from either disease alone. The plants shown in Plate 6 also illustrate the double infection described. In these plants the symptoms of both foliage troubles were easily recognized. There is the mottling and wrinkling of leaflets, shortened petioles, and crowding of leaflets typical of mosaic, and, in addition, the rigid texture, the rolling of leaves, and the "staring" habit characteristic of leafroll.

Table I furnishes evidence on several points as follows: (*a*) The data tend to substantiate the statement made in another portion of this paper that spindling-sprout is correlated with leafroll but not with mosaic. It will be noted in this connection that mosaic symptoms are recorded as associated with leafroll in some of the plants from spindling-sprout tubers, but that no cases of leafroll are cited as occurring in the plants from normal sprouts. It is not to be assumed that symptoms of leafroll do not occur in plants from tubers with normal-appearing sprouts, but it is evident that there is a correlation between spindling-sprout and leafroll and, further, that this is independent of the mosaic symptoms. (*b*) Tubers with normal sprouts produced either mosaic or healthy plants and in general larger yields than tubers with spindling sprouts. The hill yields of the tuber units show a fairly uniform decrease, being largest in hills from bud-end seed pieces and smallest in hills from stem-end pieces. This was true not only of the leafroll and mosaic but of healthy tuber units.

TABLE I.—*Yields by tuber units from spindling-sprout and normally sprouted seed*

Record No. (1921).	Condition of sprouts.	Yields in grams of tuber unit hills—					Total.	Average per hill.	Condition of plants (1921).
		a.	b.	c.	d.	e.			
75 (16-1)	Spindling	184.1	116	67.5	93.7	45	506.3	101.2	Leafroll.
76 (16-2) [a]	do	224	182.8	194.5	124.5	256	982.4	196.4	Do.
77 (16-3)	do						231.7	77.2	Leafroll and mosaic.
82 (18-2)	do	111	67.3	39	40.5		258	64.5	Very weak leafroll and mosaic.
85 (19-2)	do	117	116				233	106.5	Leafroll and mosaic.
86 (19-3)	do	60	35	26.5	23.5		145	36.2	Leafroll.
87 [b]	do	87	41.5	31.4			159.9	53.3	Leafroll and mosaic.
89 [b]	do	139.9	79.4				219.3	109.6	Do.
78 (17-1)	Normal						659	164.7	Mosaic (medium).
79 (17-2)	do						760.8	253.6	Healthy.
80 (17-3)	do	513	421	362	175.7		1,471.7	367.9	Do.
81 (18-1)	do						958.7	191.7	Do.
83 (18-3)	do						232	46.4	Mosaic.
84 (19-1)	do	327	276.5	263	184.5		1,051	262.7	Do.
88	do	282	230	206	115		833.3	208.3	Healthy.
90	do	304.8	254				588.8	279.4	Mosiac.

[a] It is possible that an error in placing the seed pieces is responsible for the larger yield of hill e in this sample, since such a result is not in harmony with those from the other tuber units.
[b] Tubers showed net-necrosis when cut.

VARIATION IN THE PROGRESSIVE DEVELOPMENT OF MOSAIC SYMPTOMS

Schultz and Folsom (*11, p. 316*) recognize and describe five stages or degrees of severity of mosaic symptoms—"slight," "slight plus," "medium," "medium plus," and "bad." The writer, while agreeing fully as to the significance and appropriateness of these terms, has, however, used the slightly different terms, "mild," "medium," and "extreme," respectively, for the conditions described by Schultz and Folsom as "slight," "medium," and "bad." "Mild" mosaic in the present paper indicates faint mottling with approximately normal development of foliage; "medium" refers to a condition in which there is distinct mottling and some wrinkling and reduction in leaf area, and "extreme" is used in connection with the stage in which there is marked wrinkling and curling of leaves and a dwarfed or conspicuously spindling habit of the plant. The results of Schultz and Folsom (*11, p. 317*) indicate that mosaic in northern Maine "does not necessarily change much from year to year in any diseased stock after the first appearance of the effects of infection." The results of the present investigation in part seem to agree and in part to differ with the foregoing statement. That is to say, it has been found that in Vermont mild mosaic may in some cases persist as mild mosaic for several seasons in a certain stock without marked change, but that from one season to the next sudden changes also occur, as from mild to extreme, or even from a condition noted as "healthy but adjacent to mosaic hills" to a condition of extreme mosaic as described in the succeeding paragraphs.

As a result of observations on many plantings of tubers of known history both in the greenhouse and field, it was noted that much variation exists in the progressive development of mosaic infection from year to year. In certain cases a condition of mild mosaic persists in the progeny plants through one or more subsequent seasons with comparatively little change, while in other cases the increased seriousness of the disease as evidenced by the foliage symptoms is very marked between one season and the next. In the first instance tubers from plants exhibiting a mild degree of infection produced the next season plants showing little, if any, increase or change in the seriousness of the symptoms (Pl. 2, A 2; D), while in the latter case tubers from other plants with very similar external symptoms produced the next season plants showing, even in the earliest stages of development, extreme mosaic symptoms (Pl. 2, A 1; C).

In illustration of the first condition described is cited the history of some tubers of the Green Mountain variety secured in 1919 from L. P. Boyd of Whitingham, Vt. The plants from which these tubers were taken were of large growth with leaves showing faint mottling but no wrinkling. There was apparently no reduction in area of leaf surface on account of the disease. Tubers from these hills planted in the experimental plots in 1920 gave plants showing only a faint mottling and no wrinkling or dwarfing throughout the season. Tubers from the 1920 plants in the field plots were in turn planted in the greenhouse in the winter of 1920–21, and the foliage produced showed faint mottling, slight wrinkling, and symptoms of leafroll. Since the plants in the field during the season of 1920 were adjacent to leafroll hills, there was sufficient opportunity for the acquisition of this infection. There is seen, then, in the foliage of three successive generations of this particular lot (III–18–2–1919) only a slight increase in the seriousness of mosaic symptoms,

and that in the third season. No record was obtainable of the disease status of this sample previous to 1919.

Referring now to the other condition mentioned previously—namely, that in which there seemed to occur a marked increase in seriousness of infection from one season to the next, the following cases are cited: (*a*) It was observed in plants just emerging from the soil in the greenhouse bench that certain ones exhibited marked wrinkling and mottling of leaves at this early stage (Pl. 2, B and C), while others, which later developed typical though mild mosaic symptoms, showed no mottling or wrinkling at the corresponding period of development (Pl. 2, D). The plants shown in Plate 2, C and D, are both from parentage indicated as mild mosaic but not from the same hill. (*b*) Again, a case of extreme mosaic is observed in a very young plant which is from a parentage indicated as "healthy but adjacent to mosaic hills" (Pl. 2, B).

In Plate 2 the potted plants A, 1 and A, 2 are grown from the same corresponding tubers as the plants shown in C and D. A, 1 and C from the same tuber developed early the extreme type of mosaic from mosaic mild ancestry, while A, 2 and D developed mild mosaic symptoms from an ancestry exhibiting the same type of infection.

The writer has at hand no definite evidence upon which to base an explanation of the differing types of mosaic infection just described. There may be some weight accorded to the suggestion that certain plants possess a factor of resistance to mosaic infection which is absent in other plants. It is also conceivable that the divergencies in the type and seriousness of symptoms observed may be due to differences in amount of infective material introduced by aphids or other agencies. Another and perhaps more plausible explanation is that in the plants exhibiting the mild and extreme symptoms, there may occur what are in effect distinct types or strains of mosaic possessing sufficient individuality to account for the persistence of either through successive generations of the host plant. The sudden development of the extreme type in such plants as are illustrated in Plate 2, B, and the continuation of the mild type in Plate 2, D, may be considered as furnishing some support for this argument. It is to be hoped that further investigations will throw light upon this point.

SPINDLING-SPROUT AND LEAFROLL

In 1916 Stewart (*13, p. 351*) in a discussion of the results of his "Observations on Some Degenerate Strains of Potatoes," summarizes his conclusions in part as follows: "Although spindling-sprout symptoms appeared occasionally in the tubers of plants affected with mosaic, leafroll, and curly-dwarf, they were too infrequent to warrant the conclusion that spindling-sprout is correlated with any of these three diseases." He further adds, "sprouting tests of tubers from leafroll, curly-dwarf, and mosaic plants show that spindling-sprout is not a symptom of these diseases." In general, the present investigation has shown that spindling-sprout, while it is not, as a rule, a symptom of curly-dwarf or mosaic, is a consistent symptom of leafroll and often also of net-necrosis. Stewart's procedure in noting the sprout condition of tubers from plants of certain observed foliage symptoms of the previous season, seems to the writer to be not so dependable with reference to the information sought as that which takes into account as well, and indeed, primarily, the foliage condition of the progeny plants. It must frequently occur

in plots or fields where both mosaic and leafroll are common that vines receive infection during the season which does not develop into external symptoms until the following season. For example, plants with apparently normal and healthy foliage throughout the season nevertheless produce tubers which in the following season develop plants with various stages of mosaic or leafroll. Evidently the sprouts of such tubers would have significance with reference to the foliage condition of the plants which they produced rather than of the plants from which they came. Further, in the case of net-necrosis of the tuber it has been shown by Schultz and Folsom (*12, p. 65–71*) that this condition suddenly appears and often as suddenly disappears so far as the symptoms are concerned, and that in its correlation with leafroll it depends upon "recency of infection." Consequently, with reference to this trouble also, the symptoms of the plants resulting from net-necrosis tubers are of more significance than those of the plants producing them.

Blodgett, Fernow, and Perry report in an abstract on "Testing Seed Potatoes for Mosaic and Leafroll" (5) that "tubers indexed as leafroll grew sprouts generally thinner than healthy potatoes but not always of the extreme spindling-sprout type," and state that by a preliminary sprouting method all but 3 per cent was removed from two lots containing 50 per cent of leafroll. These writers also secured partially successful results in eliminating mosaic and leafroll from seed stock by growing one seed piece from each tuber as an early crop in the field previous to planting the main crop.

In the spring of 1921 about 60 hills were planted in the experimental plot with seed pieces from tubers which had sprouted in storage and whose sprouts showed a more or less spindling condition. Of these, 20 hills were of the Burbank variety and 40 hills were Green Mountain. The 20 hills of Burbank (No. 91, 1921) planted with seed pieces from tubers all of which had developed spindling sprouts showed 100 per cent characteristic leafroll. A control row of 26 hills (No. 92, 1921) of the same variety and lot but planted with tubers which had apparently normal sprouts, produced 92 per cent healthy hills and 8 per cent leafroll hills. Three of the leafroll hills from No. 91 (1921) and one healthy hill from No. 92, (1921) are shown in Plate 3.

The 40 hills of Green Mountains were planted in single tuber units with tubers selected from various samples of the preceding season, these tubers having developed in storage sprouts of more or less marked spindliness. Without exception, each hill of every tuber unit developed leafroll in varying degrees of seriousness. All the tuber units were planted in the same order, the seed pieces from the bud end or apex first and the others progressively in order to the stem end. With a large degree of uniformity the plants from the bud-end pieces were larger, more vigorous, and more productive, while a rather uniform decrease in size and productivity characterized the plants from the middle and stem-end pieces. Plate 4, A, B, C, and D, shows four of the five plants of tuber unit series 75 (16–1) illustrating characteristic leafroll symptoms and gradual decrease in size. The photographs of these hills were taken with the camera at the same distance in each case. All of the plants were rather small, the smallest being about 8 inches in height. In Plate 5, A, 75, may be seen the sprouted tuber from which these plants were grown. It will be observed that there is one rather vigorous sprout at the apex and that the remaining sprouts are more or less spindling, a condition very common in connection with this type of degeneracy. In

general, spindliness tends to begin in the sprouts at or near the stem end of the tuber, one or more sprouts at the apex often remaining normal in size and appearance except in the most extreme cases, such as is shown in Plate 5, D, 85, where all the sprouts have become spindling. That the varying degrees of spindliness of sprout are correlated with differences in vigor and productivity of the resulting plants is illustrated in Plate 4, A, B, C, D; Plate 6, A, B, C, D, E; and Tables I and II.

TABLE II.—*Yields in grams from average hills of Burbank plants which exhibited extreme and mild leafroll symptoms and normal foliage, respectively*

Record No. (1921).	Condition of sprout.	Yields from average hills—				Total.	Average per hill.	Condition of plants.
		1	2	3	4			
91a	Spindling	120.4	63.2	0	0	183.6	91.8	Extreme leafroll.
91b	do	329.9	195.9	0	0	525.8	262.9	Mild leafroll.
92	Normal	835.7	584	591	613	2,624.5	656.1	Healthy.

In Table II, No. 91a and 91b included tubers selected for very much marked spindliness of sprout. As indicated previously, all the plants from these tubers developed characteristic leafroll. The leafroll symptoms, however, varied in seriousness and the plants showing extreme symptoms, being much dwarfed, were indicated as 91a, while those of larger growth but still with well-marked leafroll characters, were designated as 91b. Average hills from each of these groups were selected and the yields weighed, with the results given in the table. For comparison, four average healthy hills from lot 92 (1921) were selected and the yields weighed. The results correspond closely to expectation as governed by the condition of the foliage. Wide variation in yields between the two types of leafroll appears, and a difference nearly as great between mild leafroll and healthy hills.

CORRELATION OF NET-NECROSIS WITH SPINDLING-SPROUT AND LEAFROLL

In cutting the tubers of lot 91 (1921), referred to in preceding paragraphs, it was noted that every tuber showed typical net-necrosis of the sort illustrated in Plate 6, A, affecting a portion of its tissues. These tubers had been selected for investigation because of the extreme spindliness of the sprouts which had developed in the storage cellar during early spring. In the experimental plot the plants from these tubers developed 100 per cent leafroll. Many of the hills were very weak and some seed pieces produced no plants at all. In lot 92 (1921) of the same variety selected for normal sprouts no net-necrosis was found, and of this lot, as noted previously, 92 per cent of the plants were healthy and 8 per cent showed leafroll. A tuber unit series from a net-necrosis tuber is illustrated in Plate 6. Three of the four seed pieces of this tuber showed necrosis of the cortical region and from near the edges of the necrotic tissue in two of the pieces sprouts which were thin and spindling had developed. The seed piece from the apex of the tuber developed two sprouts apparently normal, but the plant from this piece, as well as those from the pieces showing necrosis, developed both leafroll and mosaic symptoms. The occurrence of the mosaic symptoms in this instance, as in some others observed, is be-

lieved to be incidental so far as its connection with net-necrosis is concerned. A study of Table I will reveal the fact that leafroll resulted in every case of spindling-sprout, either alone or associated with mosaic, while there was no case of spindling-sprout observed when mosaic alone appeared in the resulting plants. On the other hand, numerous cases of mosaic of both the mild and extreme types were recorded in plants from tubers with normal sprouts. Similar conclusions are reached with reference to net-necrosis—that is, that while both mosaic and leafroll symptoms have been noted in plants from net-necrosis tubers, in no cases have such tubers resulted in plants exhibiting mosaic symptoms only. Net-necrosis, therefore, of the type discussed and illustrated in the present paper, is definitely and closely correlated with spindling-sprout of the tuber and with leafroll of the plant.

TABLE III.—*Showing correlation of spindling-sprout and net-necrosis of the tuber with leafroll of the foliage*

Lot No.	Number of tubers.	Condition of sprouts.	Net-necrosis.	Number of hills.	Condition of foliage.
87.........	1	Spindling....	Present....	4	Leafroll and mosaic.
89.........	1	.....do.......	.....do.....	3	Do.
91.........	8	.....do.......	.....do.....	20	Leafroll.
96.........	1	.....do.......	.....do.....	4	Do.
108.........	3	.....do.......	.....do.....	13	Do.
111.........	2	.....do.......	.....do	4	Do.

APICAL DOMINANCE

Appleman in an article entitled "Some Factors Influencing the Vitality of Seed Potatoes" (*4*) in Maryland Farmer, volume 5, No. 16, 1921, states that "good normal tubers will as a rule sprout first from the seed or apical end," and also that "lack of apical dominance is a sign of low vitality in the tuber or in other words of its inability to produce vigorous plants." In another publication (*3, p. 80*) this author says: "The greater vigor of the terminal shoot may be due to greater inherent strength of the terminal bud or it may be merely apparent because the ternimal shoot inhibits the growth of the lateral ones. The latter view suits best the requirements regarding the growth of buds on the potato tuber." He further states that while, under uniform conditions, terminal buds ordinarily grow out first, still if the basal eyes are more favorably situated with respect to the action of external agents the suppression of their growth by terminal buds may be entirely overcome. These facts are understood to be true only of the first crop of sprouts from a tuber.

In connection with the studies reported in the present paper many observations have been made as to the manner of sprouting of diseased and healthy tubers ,some of which have a bearing upon the matter of apical dominance.. Tubers with normal sprouts from the apex and with the other eyes dormant were selected for planting and also those with normal sprouts from various eyes scattered over the tubers. Table IV gives the results of the observations upon the plants grown from these tubers.

TABLE IV.—*Showing relation between distribution of sprouts and foliage condition of progeny plants*

Lot No.	Number of tubers.	Distribution of sprouts.	Number of plants.	Condition of plants.
61	1	Clustered at apex	4	Medium mosaic.
62	1	.....do	4	Healthy.
63	1	.....do	2	Do.
65	1	.....do	4	Extreme mosaic.
66	1	.....do	3	Do.
67	1	.....do	4	Do.
78	1	.....do	4	Do.
79	1	.....do	3	Healthy.
80	1	.....do	4	Do.
57	1	Scattered	6	Medium mosaic.
58	1	.....do	5	Mild mosaic.
59	1	.....do	3	Medium mosaic.
60	1	.....do	3	Extreme mosaic.
64	1	.....do	5	Mild mosaic.
68	1	.....do	6	Medium mosaic.
69	1	.....do	5	Healthy.
70	1	.....do	4	Mild mosaic.
81	1	.....do	8	Healthy.
83	1	.....do	5	Mild mosaic.
84	1	.....do	4	Healthy.
88	1	.....do	4	Medium mosaic.
90	1	.....do	2	Extreme mosaic.

The results of these limited observations do not show either apical dominance or the lack of it in the tubers to be consistently correlated with any condition as to health or disease in the resulting plants. Tubers with sprouts clustered at the apex produced both healthy and mosaic plants and the same results were observed in the case of tubers with sprouts not confined to the bud end or apex. In Plate 5, B, 78, 79, 80, are shown tubers with normal sprouts clustered at the apex, and in C, 81, 83, and D, 84, of the same plate are shown tubers without this apical dominance. By referring to Table I it will be seen that of the tubers showing apical dominance, No. 78 produced mosaic plants, while No. 79 and 80 gave healthy plants, also, that of the tubers not exhibiting this dominance, No. 81 gave healthy plants while No. 83 and 84 gave mosaic plants.

In agreement with the statement of Appleman referred to above, the present observations indicate that apical dominance may be suppressed when basal eyes are situated more favorably for germination than terminal ones, so as an index of vigor in tubers this characteristic would be subject to limitations. Moreover, as an index of vigor it is subject to the further limitation that tubers with distinct apical dominance may develop mosaic and even mosaic of the extreme type, as in tubers 65, 66, 67, and 78 in Table IV.

Referring again to the data in Table IV, the records show that in the germination of tubers 61, 62, and 63 the plants from bud-end seed pieces started more promptly and grew better than the plants from the stem end of the tubers. The records further show in a limited number of cases examined that, regardless of disease condition, when there is marked apical dominance of sprouts, the plants from the apex grow more vigorously and those from the stem end less so, while in cases of

tubers with scattered sprouts, all apparently of equal vigor, the plants from the several seed pieces are approximately uniform. It seems to be true that a degree of apical dominance is shown in the material studied, in the large size and yields of hills from apical buds. The fact that should be emphasized, however, is that this dominance is not an index of freedom either from mosaic or leafroll.

The writer's conclusion with reference to the bearing of apical dominance upon the present problem is that the appearance of the sprouts as to normal thickness, branching, etc., is a better index of the healthy or diseased condition of the tuber and of the resulting plants than the distribution of the sprouts on the tuber; and further, that the data so far collected fails to support any consistent correlation between the distribution of sprouts on the tubers in storage and mosaic of the resulting plants.

SUMMARY

(1) In instances both of serious mosaic and serious leafroll infection, germination of the seed pieces and growth of the shoots are markedly retarded.

(2) (*a*) In the strains of Green Mountain potatoes under observation in these investigations the same plants frequently exhibited both mosaic and leafroll infection.

(*b*) All the data secured support the thesis that there is uniformity as to condition of health or disease in all the plants from a single tuber and also in all the tubers from a single hill.

(3) Marked variation in the progressive development of mosaic infection with the suggestion of differing strains of mosaic virus were indicated in the germination of tubers of known ancestry and in the observation of several successive generations of plants from known sources.

(4) (*a*) Spindling-sprout of the tubers is shown to be, in the varieties of potatoes studied, a consistent symptom of leafroll, but not of mosaic.

(*b*) Net-necrosis, of the phloem-necrosis type, is correlated in the tubers with spindliness of sprout and seems to be a consistent symptom of leafroll. The necrosis symptoms are not persistent in the progeny tubers.

(*c*) Yields of plants from spindling-sprout tubers were much reduced in comparison with those of plants from tubers with normal sprouts, and hills exhibiting extreme leafroll yielded far less than those with mild leafroll symptoms.

(5) Apical dominance in sprouting which has been held to be an indication of vigor in tubers was found to be associated both with the development of mosaic in the foliage and with a healthy condition of plants, and conversely, the lack of apical dominance was associated with the production of healthy as well as mosaic hills.

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PLATE 1

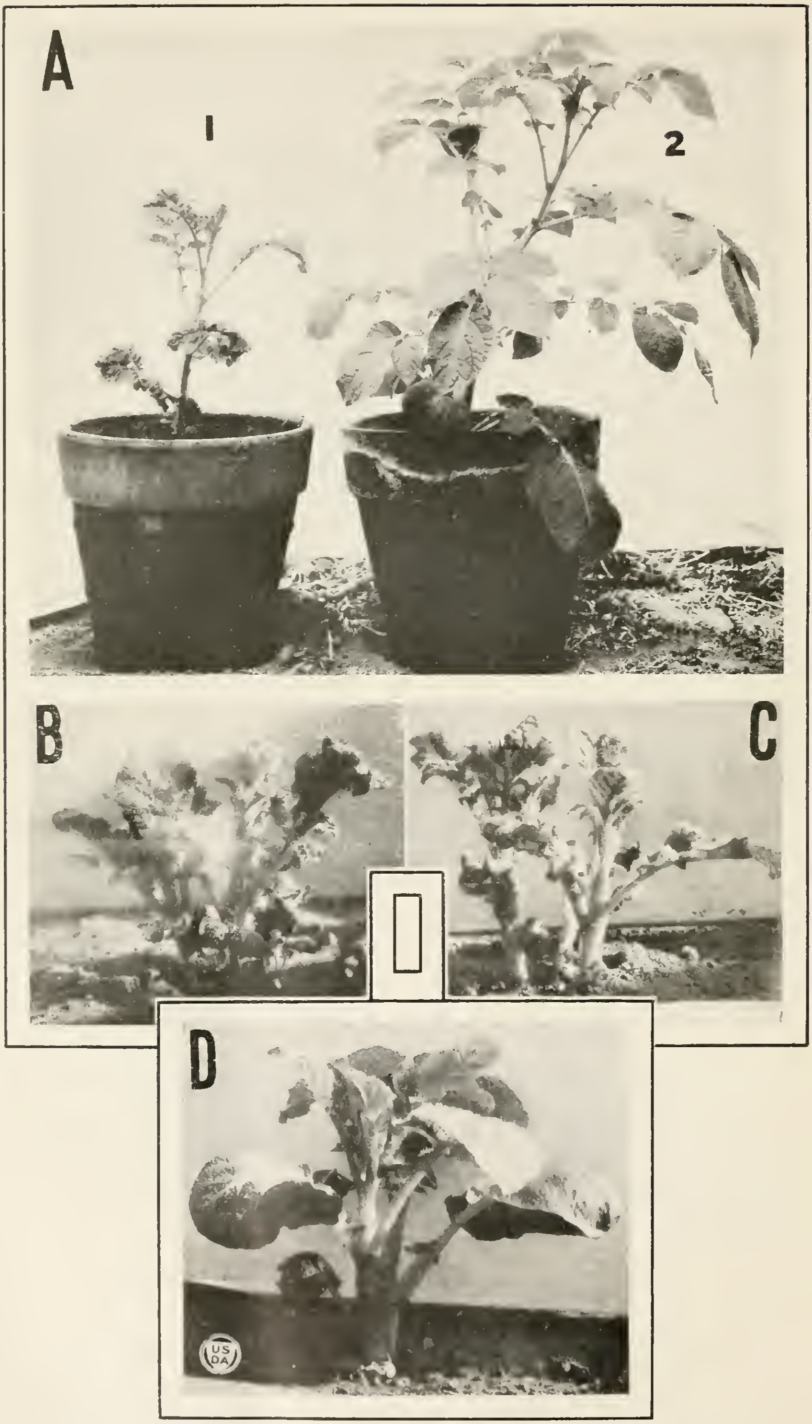
A.—Two potted plants of the Green Mountain variety and sprouted seed pieces from the same corresponding tubers. On the left the sprouts of the seed piece are retarded in growth and show several brownish lesions, and the potted plant exhibits symptoms of extreme mosaic; on the right the seed piece has a sprout approximately normal, and the plant exhibits mild mosaic.

B.—A potted plant with symptoms of extreme mosaic and sprouted seed piece from the same tuber. The sprout exhibits retarded growth though not as marked as in A. The seed tuber was from a parentage described as "healthy but adjacent to mosaic plants."

C.—A potted plant of the Green Mountain variety exhibiting symptoms of both leafroll and mosaic. Irregular brownish areas appeared in the leaves and on the stems, and premature defoliation occurred.


A


B
C


A
1
2
B
C
D
US
DA

266[3]

PLATE 2

A.—Plants of the Green Mountain variety exhibiting two types of mosaic symptoms, grown in greenhouse. 1, showing marked spindling habit and reduced leaf surface accompanied by wrinkling and mottling, the extreme type. 2, foliage nearly normal as to development but with the characteristic mottling, the mild type. Both plants are from mild mosaic parentage but from different hills.

B.—Young Green Mountain plant showing early the symptoms of extreme mosaic. Grown in greenhouse bench (1920–21) from parentage designated as "healthy but adjacent to mosaic hills," in the experimental field in 1920.

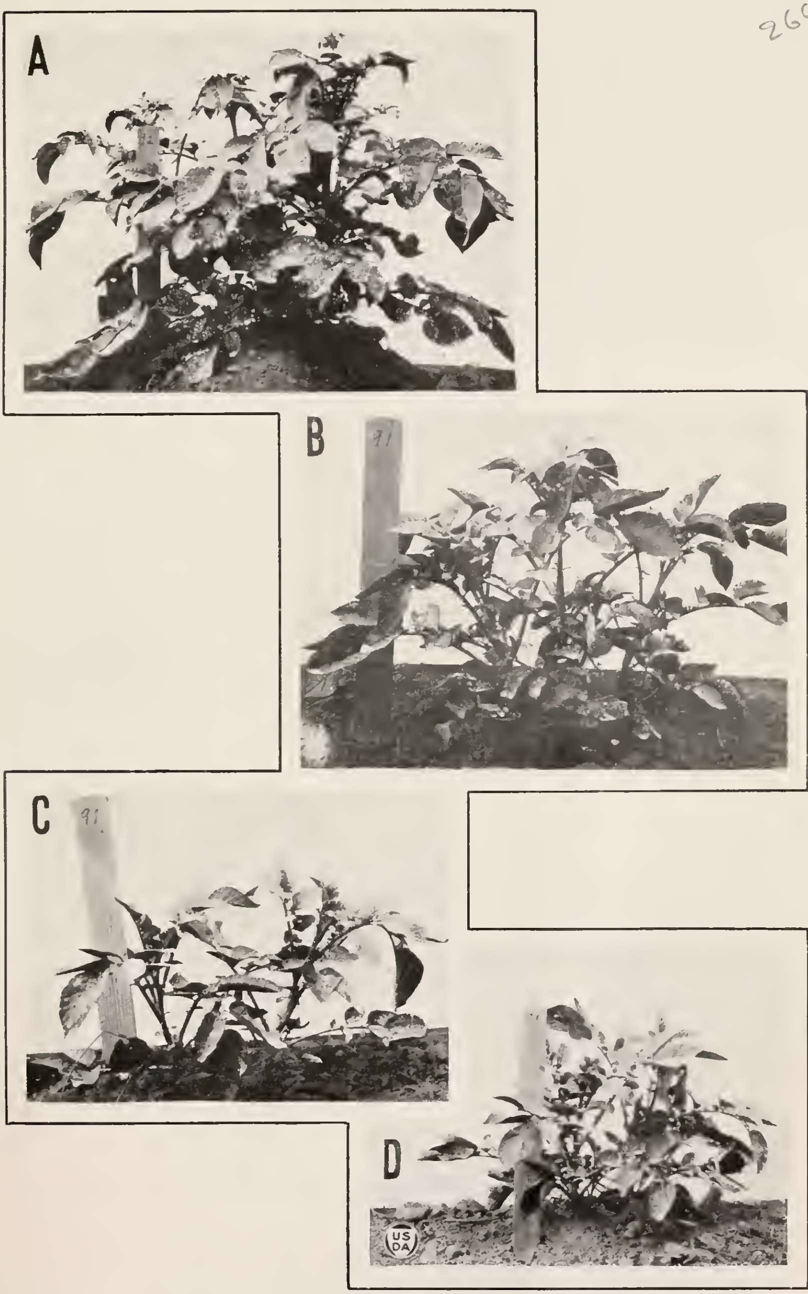
C.—Young Green Mountain plant of the extreme type, developing the symptoms very early in the greenhouse bench (1920–21), from parentage designated as mild mosaic in the experimental field in 1920. From the same tuber as A, 1.

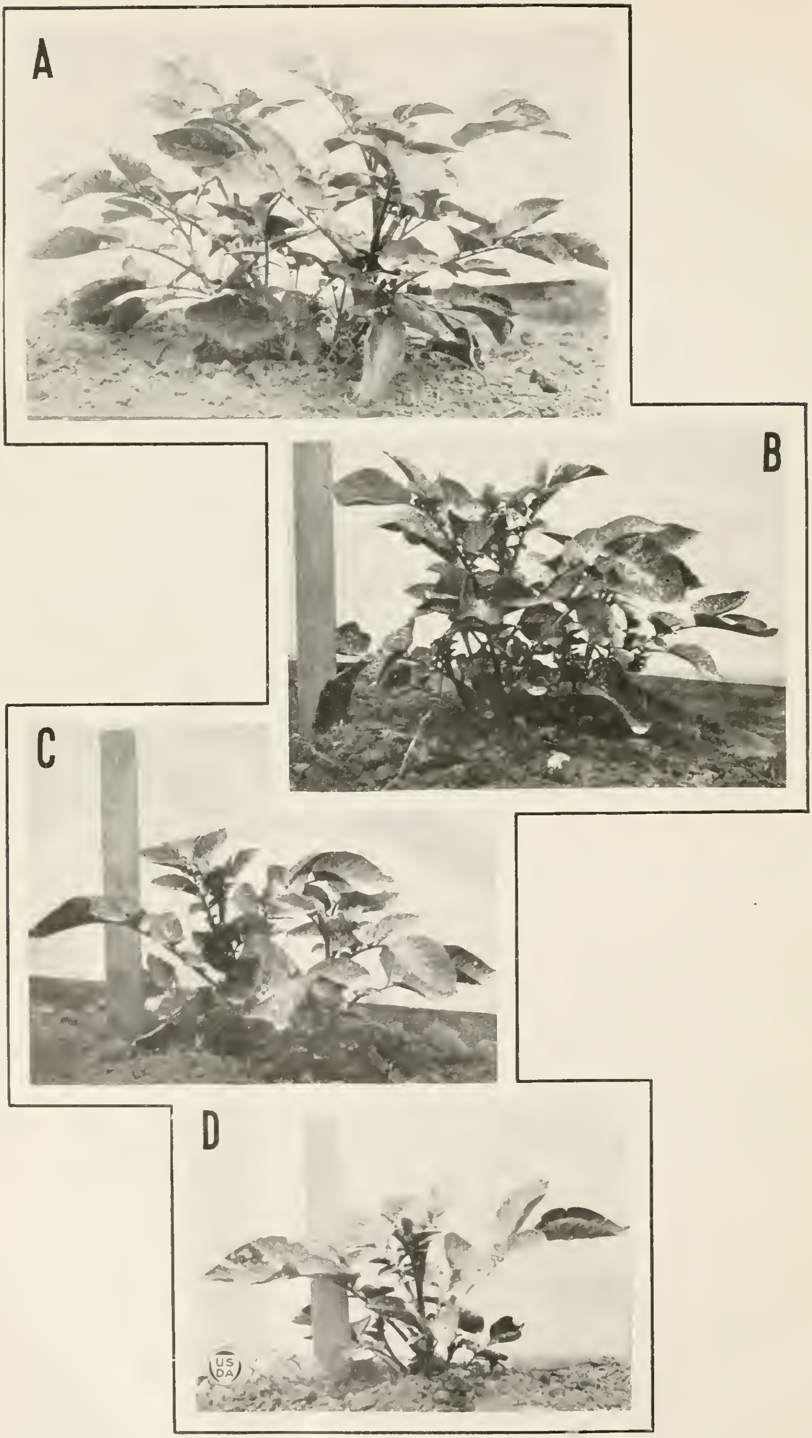
D.—Young plant apparently healthy at first but later developing mild mosaic symptoms. From mild mosaic parentage and from the same seed tuber as A, 2.

PLATE 3

A.—Healthy hill of Burbank variety (lot No. 92) grown from tuber with normal sprouts.

B, C, D.—Leafroll hills of the same variety as A but grown from tubers with spindling sprouts and net-necrosis (lot No. 91). All plants of this lot developed characteristic leafroll.


A
B
C
D


A
B
C
D

266

PLATE 4

Four hills from tuber No. 75 (16-1) planted in the order A, B, C, D, with seed pieces taken as follows:

A.—From the bud end.

B, C.—From the middle.

D.—From the stem end of the tuber.

All hills exhibit characteristic leafroll. The seed tuber developed spindling sprouts from several eyes. See plate 5, A.

PLATE 5

Green Mountain tubers which sprouted in the storage cellar in the spring.

A.—Tubers 75, 76, and 77 illustrate a condition, frequently observed, in which there is one apparently normal sprout at the bud end, the remaining sprouts being more or less spindling. All hills from these tubers showed leafroll or combined leafroll and mosaic symptoms.

B.—Tubers 78, 79, and 80 with normal sprouts clustered at the bud ends. Tuber 78 produced mosaic plants while 79 and 80 gave plants entirely healthy throughout the season.

C.—Tuber 81 produced healthy plants; 82, very weak plants showing both leafroll and mosaic (note the spindling sprouts toward the stem end); and 83, mosaic plants.

D.—Tuber 84 with normal sprouts produced medium mosaic hills, while tubers 85 and 86, with spindling sprouts, produced very weak plants showing leafroll symptoms.

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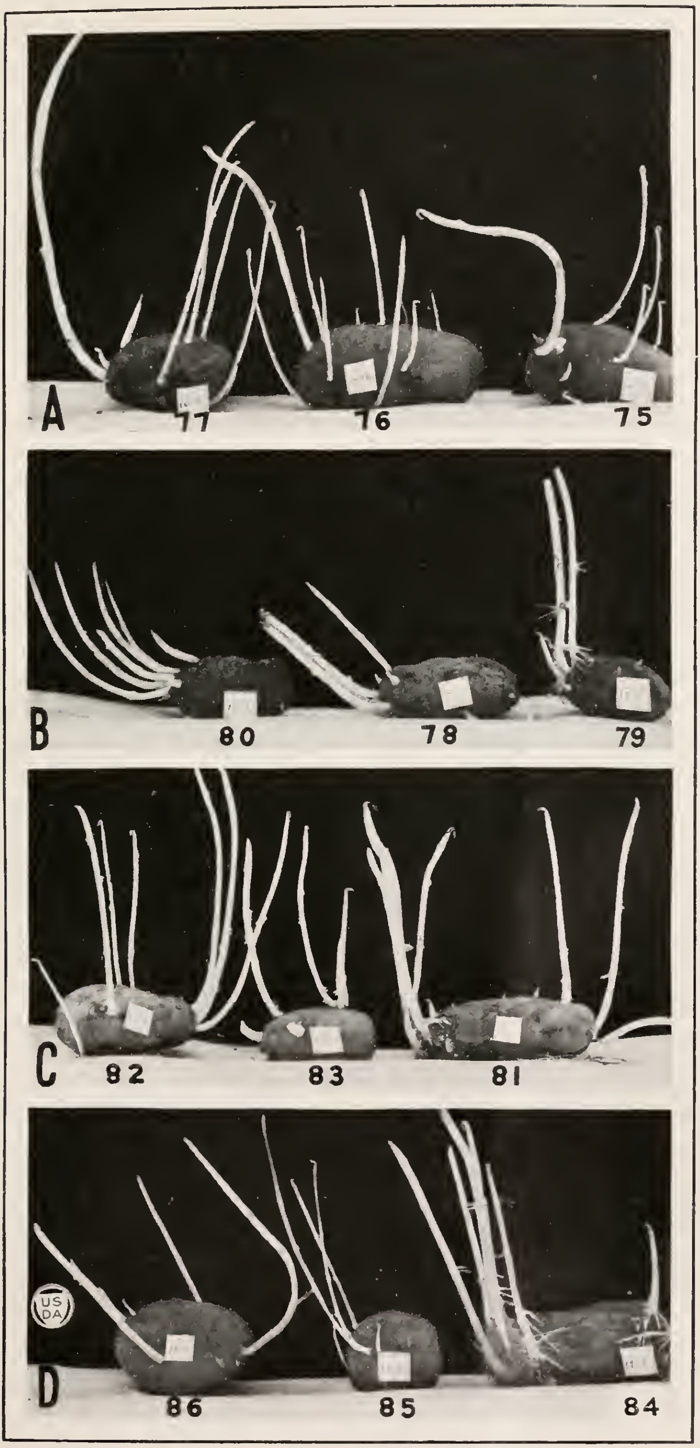

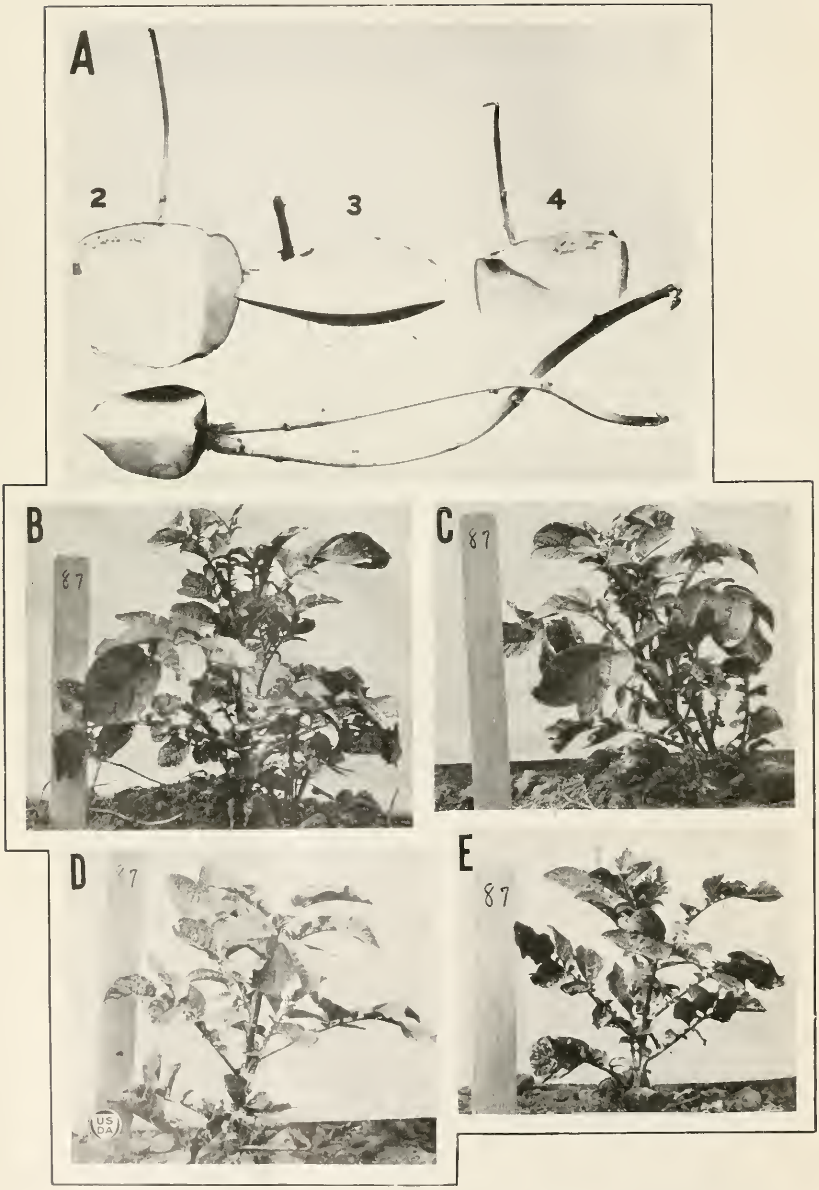
A
2
3
4
B
87
C
87
D
E
87

PLATE 6

A.—Seed pieces of tuber No. 87 exhibiting spindliness of some of the sprouts and net-necrosis of the tuber.

B, C, D, and E.—Plants grown from seed pieces 1, 2, 3, and 4, respectively. Both leafroll and mosaic symptoms developed in these plants. A gradual decrease in size and vigor is noted in the plants from the middle and stem-end pieces as compared with that from the bud end. The bud-end piece showed neither necrosis nor spindliness of sprout, but produced, like the others, a diseased plant.

COMPARATIVE STUDY OF PHYTOPHTHORA FABERI ON COCONUT AND CACAO IN THE PHILIPPINE ISLANDS.[1]

OTTO AUGUST REINKING[2]

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INTRODUCTION

Each year in the Philippine Islands, severe losses result from coconut budrot and from blackrot and canker of cacao. In 1919, the writer (7)[3] produced evidence indicating that the organisms causing both diseases were identical. These studies have been continued, and detailed cross inoculation experiments, comparisions of parallel cultures, and more complete physiological and morphological studies have been made.

Coconut budrot is a serious disease of the coconut (*Cocos nucifera*), affecting the single terminal bud of the tree. Throughout the world various organisms, including bacteria and fungi, have been considered responsible for the disease. In the Philippine Islands there are apparently two types of budrot—the really infectious type caused by a Phytophthora, and a secondary type following some injury such as beetle injury and caused primarily by the invasion of bacteria in the weakened tissue. The earliest symptoms of the infectious type are usually the production of rows of dark-brown spots on the newly unfolded leaves. Later developed leaves then show a severe leaf blighting. Finally, the central group of folded leaves dies and turns brown, indicating the death of the growing point. The bud may not be killed until after 12 months from the time of the first leaf spotting. The usual period, however, is from 2 to 3 months. The fringe of older, healthy, green leaves remains on the tree until it dies a natural death. Infected trees are stunted (Pl. 4, B) and the nuts do not develop. Internal symptoms are characterized by a browning of the folded leaves above the growing point. A white mycelial felt may or may not be present on these young leaves. From the growing point, downward and to the sides, the organism advances for a short distance into the woody parts, where the limits of its advance are marked by a dark red to brownish line (Pl. 1, A). In advanced cases, after the entrance of bacteria, the soft region about the growing point is changed to a semiliquid, ill-smelling mass.

The blackrot of pods and the canker of cacao (*Theobroma cacao*) is widely distributed throughout the world in the cacao growing regions. In the Philippines, it has been reported as causing severe losses (*6, p. 192–196*). The fruit is attacked at any stage of its development. Usually the greatest damage is done to the young fruits. At first a minute black spot is developed on the infected part. This spot gradually

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[2] Plant pathologist of the United Fruit Co. in Honduras and Guatemala.

[3] Reference is made by number (italic) to "Literature cited," p. 284.

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enlarges until the pod becomes blackened. In excessive damp weather, a mass of mycelium with conidia may be produced on the surface of diseased fruit (Pl. 2, B). The mycelium invades the rind, passing into the seed. Diseased pods may fall or remain on the tree, drying up and becoming mummified. Flowers and stems may be attacked and killed by the fungus. The canker is found on young twigs, older branches, and the trunks of trees. Diseased twigs are characterized by browning of the leaves, dying of the tips, and shrivelling of the diseased wood. The first evidence of a canker is the appearance of a darker than normal color on the bark. The infected area may then turn brownish. A shrinking of the diseased area and a definite line of demarcation is usually produced, showing the limits of the infection. In advanced stages the bark may exude an amber fluid and later may crack and scale. The infection has been observed to spread from diseased pods into the branches or trunk. A true cankered condition is not always produced. Internal symptoms are characterized by a browning and blackening of the infected bark and wood (Pl. 2, A).

The fungus from coconut, used for the comparative study, was isolated from an infected coconut tree on March 11, 1919. The diseased tree was located in a plantation near Lilio, Laguna Province, P. I., a region in which sporadic epidemics of the disease have been recorded since 1908.

The fungus from cacao was isolated from a diseased cacao pod collected in the plantings near Los Banos, Laguna Province, P. I., during 1917. The disease is present there in a severe form throughout the year, causing most damage during the rainy season.

COMPARATIVE STUDY OF THE FUNGUS ON BOTH HOSTS

FIELD CHARACTERISTICS

On coconuts the single terminal bud of the tree is affected. A white mycelial felt may or may not be present on the young, folded, diseased leaves of the bud. Microscopic examination of the infected parts of the leaves, growing point, and surrounding tissue shows the presence of a granular, nonseptate mycelium in the intercellular spaces (Pl. 8, A). Fingerlike haustoria are produced in abundance, penetrating the host cells (Pl. 8, B). Chlamydospores, in limited numbers, have been observed in the infected buds some 60 cm. above the growing point. The writer has never observed the lateral penetration into the tender bud through the leaf bases. The penetration in all cases observed appeared to be a vertical, downward one, along the central leaf sheaths into the bud.

Diseased cacao pods may be covered with a mass of mycelium with conidia, but usually there is no macroscopic evidence of mycelium (Pl. 2, B). Conidia are produced in abundance on the surface of the fruit. Microscopic examination of the interior of diseased pods shows the presence of a granular, nonseptate, intercellular mycelium and usually a mass of chlamydospores (Pl. 8, C). In cankered areas the mycelium develops primarily internally in the diseased tissue. Rarely, as under excessive damp conditions, the hyphae are produced on the surface.

INFECTION EXPERIMENTS

The evidence given before by the writer (7) is sufficient to prove that the organism from coconut budrot can produce the disease in coconut seedlings and older trees through injuries. Further infection experi-

ments have been made with both the stab method of inoculation and inoculation of uninjured trees. In practically every instance disease was produced. Out of 17 seedlings inoculated by the stab method, 16 were severely infected (Pl. 5). Of 7 trees inoculated by merely pouring a zoospore suspension of the fungus into the bud, 6 contracted the disease and were killed. All controls remained healthy, and reisolations of the fungus were readily made from the infected trees.

One special case of inoculation of a healthy, vigorous tree under control conditions in the field bears further mention. The three trees used for this test were situated on the Faculty Hill of the College of Agriculture at Los Banos, Laguna Province, P. I. The trees, located near the writer's home, were far removed from other coconuts and were extremely healthy, being free from serious fungus attacks and insect infestation. They grew on a hillside where there was a good air movement and, consequently, a location not peculiarly adapted for the best development of the disease. The trees were approximately 3 years old at the time of inoculation. Cultures 1 month and 11 days old growing on corn meal were used for the inoculations. On November 20, 1919, rain water was poured into one culture flask and then as soon as zoospores were produced, the zoospore suspension was poured between the young folded leaves of the bud. Another tree was similarly inoculated, except that distilled water was used in place of the rain water. For a control the third tree was used. An equal amount of rain water free from the organism was poured into the bud. On December 19, 1919, the tree inoculated with the rain water suspension showed evidences of disease. The newly unfolded leaves had a series of brown spots on the leaflets forming a concentric ring across the entire leaf. The bud from all outward appearances was entirely healthy. This spotting continued to be produced on the newly developed leaves, becoming gradually more pronounced. On April 10, 1920, the spotting was severe. August 6, 1920, the bud apparently was still healthy, but the newly unfolded leaves were badly spotted. A severe leaf blight was observed on October 19, 1920, and the tree showed evidence of being stunted. The bud appeared to be somewhat weakened. On January 8, 1921, the bud was almost dead, being browned, and on January 25, 1921, it was dead. The other two trees were healthy and showed a marked increase in growth over the diseased tree (Pl. 4, B). A typical case of budrot was produced in the infected tree (Pl. 3, A). Under field conditions in localities where epidemics of the disease are severe, the trees succumb faster, usually within two or three months from the time of infection. The inoculated tree was finally cut down and a longitudinal section through the bud and growing point showed the disease to be typical of the naturally infected cases of budrot (Pl. 4, A). The control, as well as the tree inoculated with a zoospore suspension in distilled water, remained healthy.

Besides the coconut palm (*Cocos nucifera*), other members of the Palmae were also successfully inoculated with the organism, and disease was produced in a severe or medium form. The stab method of inoculation was used. A stab with a sterilized scalpel was made through or near the growing point and then the fungus was inserted. In each case the fungus was recovered from the diseased trees. The controls all remained healthy.

The following seedlings were killed by the fungus attack: *Archontophenix alixandraeae, Dypsis madagascariensis, Livistona rotundifolia, Normanbya merrillie. Pinanga insignis.*

Livistona rotundifolia appeared to be the most susceptible.

A medium to severe infection was produced in the seedlings *Phoenix dactylifera* and *Ptychosperma mearthurii*.

Evidence previously given (7) has also proved conclusively that the Phytophthora isolated from cacao will produce a typical case of coconut budrot in injured and uninjured trees (Pl. 3, B). The fungus isolated from cacao and used for the comparative tests was also highly parasitic on the pods and branches of cacao (Pl. 2).

In order to determine whether the two fungus strains would both, in a similar manner, attack a variety of hosts, various inoculation experiments were conducted. It is realized that these inoculation tests can not alone be used to distinguish between various species of Phytophthora, but taken into consideration with the physiological and morphological characters, it is an added step toward proving the similarity or dissimilarity of two of the strains. In every instance in the inoculation experiments, reisolations of the fungus from the diseased plants were successfully made. All controls remained healthy. The results of the various cross inoculations in which cases both strains of Phytophthora, one from coconut budrot and the other from blackrot of cacao, were used, are summarized as follows:

Seedlings severely infected by both strains—
- *Annona muricata* (guanabanos, soursop).
- *Cocos nucifera* (coconut).
- *Hevea brasiliensis* (Hevea or Para rubber).
- *Theobroma cacao* (cacao).

Seedlings slightly infected by both strains—
- *Annona glabra* (annonas).
- *Mangifera indica* (mango).
- *Sandoricum Koetjape* (santol).

Fruitrot produced by both strains—
- *Carica papaya* (papaya).
- *Lycopersicum esculentum* (tomato).
- *Malus malus* (apple).
- *Theobroma cacao* (cacao).

Tuber-rot produced by both strains—
- *Solanum tuberosum* (Irish potato).

Vegetable blight produced by both strains—
- *Pisum sativum* (pea).

The coconut and cacao seedlings were readily killed by inoculation with either strain of the fungus. A severe blight of the Hevea rubber seedlings was produced. Under certain conditions a stemrot of 6 cm. developed in three days. On the soursop a rot 5 to 6 cm. in extent was formed by the attack of both strains.

The rotting of papaya fruit was brought about in the same manner by both. After the first day, the fungus mycelium was well developed on the surface and in the interior of the fruit. In the early stages a white, cottonlike growth was formed (Pl. 1, B). The diseased part was slightly discolored, being a darker yellow, especially marked at the boundary of the circular advance of the fungus. Microscopic examination showed the presence of the mycelium within the diseased tissue and the production of numerous spores on the surface. A softrot was finally formed.

Ripe and green tomato fruits were also rotted by both strains of the fungus. The appearance and rapidity of the rot was identical for both

strains. The green fruits were not so rapidly attacked. In two days there was only a slight rot produced on the green fruits, but a severe rot on the ripe ones. Later a dense growth of mycelium developed on the surface, appearing first on the ripe fruits. At the end of nine days, all fruits, both green and ripe, were severely rotted and covered with a mass of white mycelium which arose through the epidermis from within the fruit (Pl. 6, A and B). The green fruits were browned and darkened within, while the ripe fruits were only softened. Microscopic examination showed the presence of an intercellular mycelium throughout the entire fruits. A pure culture of the fungus was present on the surface with many chlamydospores and conidia.

Ben Davis apples were easily attacked and rotted by both strains of the fungus. Within five days after the inoculation through the epidermis, a rot spreading 4 cm. beneath the skin and extending to the core was produced (Pl. 6, D and E). The rotted area was brown and rather mealy. Microscopic examination showed an abundance of characteristic intercellular mycelium throughout the rotted area.

Both strains of the fungus infected cacao pods, developing the characteristic blackrot. The strain isolated from cacao appeared to be more virulent.

The rot of potatoes was produced rather slowly by both strains. Irish Cobbler and Rural New Yorker potatoes were used for the inoculations. After 6 days a slight rot developed and within 30 days the rot extended to the center of the tubers. The infected potatoes, in certain cases, showed the symptoms as recorded by Pethybridge (5) on the rotting of potato tubers by a new species of Phytophthora. No definite line of demarcation between the healthy and diseased portions was noted immediately after cutting the tubers. A blackish line at the extremity of the infection and following around with the vascular system just beneath the surface then developed. Soon after cutting the tubers, the affected portion appeared somewhat watery, and in 15 to 30 minutes a distinct pinkish to red coloration was formed in the rotted region. Later, on longer exposure to the air, the invaded parts turned a purplish black. Not in all cases of infection did the pinkish or reddish coloration develop. Microscopic examination of the infected areas showed the presence of an intercellular, nonseptate, granular mycelium.

Garden bush pea plants were readily attacked and severely blighted by both strains of the fungus. In six days after inoculation a severe blight was produced. A whitish mass of mycelium formed over the blighted parts. The mycelium was present in the intercellular spaces of the affected tissue.

The consistent similarity in the attack of various hosts by both strains of the fungus shows that in this respect there is a constant likeness. In no case, except probably with the cacao fruit, was there any difference noted in the virulence of the two. Both are omnivorous, capable of attacking a large number of different hosts.

PHYSIOLOGICAL CHARACTERISTICS

GROWTH ON VARIOUS MEDIA

The two strains grew well on a variety of media, the character of the growth being similar for each. Since the two grew alike in all particulars, a separate description of each will not be given. The following discussion applies equally well to either. The descriptions were made

from cultures growing in the ordinary laboratory light under tropical room temperatures.

Corn meal.—At first the growth on corn meal was very scant, being hardly recognized. The mycelium spread slowly and thinly in a circular area over the medium. Few, short, aerial hyphae were produced. In two to four days the growth was sparse and granular, due to the production of spores. As the culture grew older, the hyphae became more in evidence and a distinct granular appearance was formed. Finally a slight cottony, much granular growth developed over the surface of the corn meal. An abundance of conidia and fewer chlamydospores were produced.

Lima bean agar (100 gm. lima beans, 20 gm. agar, 1,000 cc. water).—Growth started with few aerial hyphae that gradually spread over the surface of the agar slant. In three days the mycelium had nearly spread over the surface. It was then characterized by being aerial, white, cottony, and granular. The granular appearance, due to spore production, was more in evidence on the walls of the glass tube at the edges of the growth. As the culture grew older, there was a gradual thickening of the aerial, mycelium mass.

Oat meal agar (100 gm. Quaker Oats, 17 gm. agar, 1,000 cc. water).—At first a few aerial hyphae developed which then gradually spread over the surface of the slant. After four days the mycelium, although still not very thick, had spread over the whole surface of the agar. Many granular bodies developed at this stage. The growth was exceedingly rapid and soon the mycelium formed a thick mass evenly spread over the agar slant, being slightly thicker at the base. As the culture grew older the hyphal mass gradually became more and more dense. Chlamydospores were produced in abundance and conidia were not so much in evidence.

Potato dextrose agar (100 gm. potatoes, 20 gm. dextrose, 15 gm. agar, 1,000 cc. water).—The first growth was characterized by being flocculent and submerged in the medium. On the second day, aerial hyphae appeared along with an increase of the submerged, flocculent growth. The aerial hyphae gradually became more dense and covered the entire slant. After five days the vegetation was dense, white, cottony, and usually thicker on the lower part of the slant. Minute granules formed by the spores were soon in evidence. Chlamydospores appeared to be produced first and in more abundance than the conidia.

RELATION TO LIGHT

In all of the cultures after exposure to the ordinary laboratory light for four days spores were produced copiously. If the cultures were kept in a dark chamber or incubator, free from light, only a few spores were formed. Sporulation was therefore hindered to a certain extent by darkness. There were apparently more chlamydospores than conidia produced in the dark. The growth in the light was more granular than that in the dark.

RELATION TO TEMPERATURE

Both strains of the fungus behaved the same in their relation to temperature. Altmann controlled temperature incubators ranging from about 4° to 32° were used for these determinations. A parallel series on potato dextrose agar plates was placed at the various temperatures and the difference in vegetative growth was determined by measure-

ments of the diameter of the mycelial mass in each case. Very slight growth for both strains was noted at 12°. The vegetation gradually increased in extent up to between 27° and 30°. From 30° and above there was a gradual decrease. The optimum growth occurred between 27° and 30°, being nearer 27°. Growth still was very good at a temperature of 32°. Below 12° no growth was noted. Neither fungus strain, however, was killed when held at an average temperature of 11.5° for 16 days, for growth took place after removal from the incubator. When kept at an average temperature of 7.5°, the minimum being about 6° for the same period, they were killed.

In all cases of growth the hyphae grew into the agar. At the lower temperatures the mycelium formed a dense, flat, surface mat with few aerial hyphae. At temperatures of 22°, and above, a tufted, serial mass of hyphae was produced, radiating out from the center. No spores were formed at the lower temperatures, between 12° and 20°. Few conidia and chlamydospores developed at the higher temperatures on the plates kept in the dark. In cases where spores formed in the dark, the chlamydospores appeared to predominate. There was a marked contrast between the growth on plates of both strains of the fungus kept in the light at a room temperature of about 22° and that on plates kept in the dark in the incubator at the same temperature. In both cases the vegetative development was approximately the same in extent. In the dark, however, the granular development of aerial hyphae was not produced and few conidia and chlamydospores formed. The growth on plates in the light was tufted, aerial, and granular, with an abundance of both chlamydospores and conidia. The development of the coconut strain is clearly shown in Plate 7, A.

Another check on the optimum temperature growth for both strains was obtained in temperature experiments on zoospore production. A suspension of spores from corn meal cultures was made in distilled and tap water. Vials containing 5 cc. of these suspensions were placed at temperatures in the Altmann incubators ranging from 7° to 32° and at room temperature exposed to the light. In no cases during these trials were zoospores produced, but an abundance of direct germination developed. The trials were run for one and three days, respectively. Very slight direct germination of the spores from either strain was noted at a temperature of 7°. Germination and growth then increased with the increase in temperature, being best between 20° and 27.5°. The optimum temperature for direct germination and growth appeared to be around 27.5°. At 30° germination and growth were not so good. The effect of light and dark at room temperatures showed no difference in the germination.

RELATION TO ACIDITY AND ALKALINITY

The relation of both organisms to acidity and alkalinity was determined by growing them on plates of potato dextrose agar and in Czapeks solution at various hydrogen-ion concentrations ranging from 1.4 to 10+ for the potato dextrose agar and from 2 to 10+ for the Czapeks solution. During the period of the experiments the room temperatures ranged between 21.5° to 27.5°, with an average temperature of approximately 23°. In both cases the vegetative growth was determined by measuring the diameter of the growth at the various concentrations. In all cases

the strains from coconut and cacao grew so nearly the same that it was impossible to note any marked difference between them.

On potato dextrose agar a distinct curve of growth for each was produced, with no growth at a hydrogen-ion concentration of 2 and below, and slight growth at 2.4. Above this concentration there was a gradual increase in the growth up to 8.8, when a decrease was noted. Between a hydrogen-ion concentration of 5.8 and 8.8 there appeared to be no marked difference in the extent of growth. Growth still took place at a concentration of 10+.

The growth between 2.4 and 3.8 was flat, dense, thick, and tough, with the production of few chlamydospores and conidia. A fluffy, dense, thick growth with few to many chlamydospores and with a smaller number of conidia was produced between 4.0 and 5.2. The mass of hyphae was still fluffy between a hydrogen-ion concentration of 5.6 and 7.8, but gradually became thinner, with a surface growth about the edges, above these concentrations. At these concentrations many chlamydospores and conidia were produced. Between 7.8 and 8.8 the aerial, fluffy growth began to disappear. Fewer spores of both kinds were formed. Above a hydrogen-ion concentration of 9 the growth was thin, flat, being on the surface of the agar, and few to no spores were formed. At 10+ it was thin on the surface, and without spores. At all concentrations except 10+ the hyphae penetrated and grew in the agar as well as upon the surface.

The optimum for growth appeared to be between a hydrogen-ion concentration of 7.4 to 7.8. Good growth was also produced up to 8.8. Slight growth took place at an acidity of 2.4. An alkalinity of 10+ did not prevent the development of either strain.

In the Czapeks solution the difference between the initial hydrogen-ion concentration and that at the end of the experiment was obtained. Control flasks of the solutions without the fungi were also kept. The growth at the different concentrations for both the coconut and cacao strains was so nearly identical that no real difference could be noted. No vegetation was obtained between hydrogen-ion concentrations of 2 and 3.2. Slight growth started at 3.4 and then gradually increased up to 9. Above this the growth gradually decreased, but still was apparent in the flasks with an initial concentration of 10+. No spores were produced in the liquid media. The optimum growth appeared to be around 7.8.

A marked change in the hydrogen-ion concentration, except in the extremely acid solutions, was noted after the fungus had grown in the media for 20 days. Between 3.4 and 3.6 the initial and final concentrations were the same. From 4.6, and above, the final concentration was less than that of the initial. The solutions with an initial concentration of from 7.8 to 10+ all were brought down to a final concentration ranging between 7 and 7.9. The controls remained the same except for a slight reduction in the concentrations above 7.8.

These tests show that both strains of the fungus were able to withstand a rather large range of concentrations from acid to alkaline. The best growth, however, appeared to be somewhat above the neutral point, between 7.4 and 8.4. The minimum hydrogen-ion concentration at which growth took place was 2.4, and the maximum was 10+. Plate 7, B and C, shows the growth at the various hydrogen-ion concentrations.

MORPHOLOGICAL CHARACTERISTICS

A comparative study from a morphological standpoint was made of both strains. Since no marked differences could be determined, one description only will be given to represent both. The descriptions of the morphological characters given before for the coconut strain by the writer (7) will be followed, but certain additional studies will be included.

MYCELIUM

The mycelium is white, producing a dense mass in pure culture. It contains many nuclei. In young cultures it is nonseptate and granular. In older ones septa may be produced during spore production. Protoplasmic streaming is common. The submerged mycelium (Pl. 9, A and B) is more or less gnarled, while the aerial is straight. Branching is abundant. The width varies from about 3 to about 8 μ. In the host tissue the mycelium is intercellular (Pl. 8, A). Numerous fingerlike forms of haustoria are produced, penetrating the host cells (Pl. 8, B). A distinct constriction is formed at the point of entrance into the cell. No cacao material was available at the time that the haustoria studies were made, so it cannot be stated with certainty that they are present in this strain. The similarity of both strains of the fungus in all other respects would lead one to believe that haustoria are also produced by the mycelium and present in the tissues of the cacao.

CONIDIOPHORES

Conidiophores are produced in great abundance in pure culture. Special culture methods must be employed to show them in their best condition. Material showing excellent conidiophore production may be obtained in sterilized Van Tieghem cells, by placing a few spores on a film of plain agar that has been spread over the flamed cover slip. Each conidiophore may bear from 1 to 15 or more conidia. A conidium is produced at the tip of a conidiophore; the latter then continues its growth by pushing the conidium to one side and produces another conidium at its tip. By a continuation of this process a bunch of spores is finally formed (Pl. 9, C and D). The conidiophores vary in size, ranging approximately from 180 to 645 μ in length, and from 3 to 6 μ in width.

CONIDIA

The conidia are produced terminally as described above. They are elliptical to ovate, and possess very prominent, raised, terminal papillae (Pl. 8, F and G; pl. 10, A and B). A short stalk may be present. The spores are pale yellow to colorless, are densely granular, contain many nuclei, and usually have a large vacuole (Pl. 12, A and B). The nuclei are clearly shown in prepared sections stained by means of the triple stain (Pl. 8, D, F, G). Older conidia usually possess more granules that are in groups.

MEASUREMENTS OF *conidia*.—The measurements conform closely to those of *Phytophthora faberi* Maubl., using the methods employed by Rosenbaum (*8*). Measurements were made from oatmeal agar cultures. The coconut culture was 9 to 11 days old, and that of cacao 16 to 19 days old. The lengths and widths of 400 spores were measured. The results are presented in Table I, which gives the class in microns and the number of conidia out of 400, both for length and width, that falls into each class.

TABLE I.—*Summary of measurements of conidia from coconut and cacao*

Class (in microns).	Number of coconut conidia in each class according to—		Number of cacao conidia in each class according to—	
	Length.	Width.	Length.	Width.
9.5 to 11.49	0	0	0	0
11.5 to 13.49	0	0	0	1
13.5 to 15.49	0	1	0	1
15.5 to 17.49	0	3	0	4
17.5 to 19.49	0	3	0	4
19.5 to 21.49	3	6	2	8
21.5 to 23.49	0	12	3	5
23.5 to 25.49	2	12	2	23
25.5 to 27.49	3	37	7	41
27.5 to 29.49	2	32	4	30
29.5 to 31.49	3	64	6	73
31.5 to 33.49	13	116	12	113
33.5 to 35.49	5	43	5	37
35.5 to 37.49	2	27	5	30
37.5 to 39.49	14	39	17	27
39.5 to 41.49	13	1	4	3
41.5 to 43.49	15	2	10	0
43.5 to 45.49	24	2	29	0
45.5 to 47.49	16	0	11	0
47.5 to 49.49	24	0	17	0
49.5 to 51.49	46	0	40	0
51.5 to 53.49	39	0	36	0
53.5 to 55.49	26	0	27	0
55.5 to 57.49	36	0	40	0
57.5 to 59.49	14	0	16	0
59.5 to 61.49	29	0	21	0
61.5 to 63.49	30	0	30	0
63.5 to 65.49	10	0	12	0
65.5 to 67.49	7	0	12	0
67.5 to 69.49	16	0	13	0
69.5 to 71.49	3	0	5	0
71.5 to 73.49	3	0	3	0
73.5 to 75.49	5	0	7	0
75.5 to 77.49	0	0	0	0
77.5 to 79.49	2	0	1	0
79.5 to 81.49	3	0	1	0
81.5 to 83.49	2	0	0	0
83.5 to 85.49	0	0	0	0
85.5 to 87.49	0	0	1	0
87.5 to 89.49	0	0	1	0
89.5 to 91.49	0	0	0	0
Total	400	400	400	400

From Table I it can be readily seen that for the coconut strain the conidia varied in length from 19.5 to 83.49 μ and in width from 13.5 to 45.49 μ. In length the majority of the spores fell into the classes between 37.5 and 63.49 μ, the average length being 52.27 μ. In width the majority fell into classes between 19.5 and 39.49 μ, the average width being 31.28 μ. These measurements made in May, 1922, on oatmeal agar show a greater range than the measurements made in 1919 on corn-meal cultures and reported by the writer (7). The average length and width, however, remained approximately the same.

For the cacao strain the conidia varied in length from 19.5 to 89.49 μ, and in width from 11.5 to 41.49 μ. In length the majority of the spores fell into the classes between 37.5 and 63.49 μ, the average length being 51.92 μ. In width the majority fell into classes between 19.5 and 39.49 μ, the average width being 30.67 μ.

The measurements of both strains show a remarkable similarity. Figures 1 and 2 present the measurements in graphic form and clearly show the close similarity of both strains.

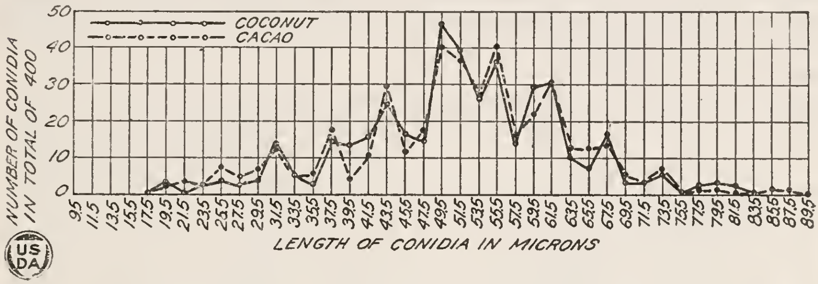

FIG. 1.—Graph showing the variation in length of conidia.

Table II gives the arrangement in classes of the ratios of the length to the width of the conidia for both strains.

TABLE II.—*Arrangement in classes of the ratios of the length to the width of conidia, showing the limits of variation*

Class (in microns).	Number of coconut spores in each class.	Number of cacao spores in each class.
0.95 to 1.04	0	0
1.05 to 1.14	1	2
1.15 to 1.24	6	7
1.25 to 1.34	31	22
1.35 to 1.44	43	48
1.45 to 1.54	57	54
1.55 to 1.64	80	59
1.65 to 1.74	53	52
1.75 to 1.84	39	54
1.85 to 1.94	28	35
1.95 to 2.04	17	27
2.05 to 2.14	24	19
2.15 to 2.24	9	8
2.25 to 2.34	4	5
2.35 to 2.44	3	2
2.45 to 2.54	2	5
2.55 to 2.64	0	0
2.65 to 2.74	0	0
2.75 to 2.84	0	0
2.85 to 2.94	0	0
2.95 to 3.04	0	1
3.05 to 3.14	1	0
3.15 to 3.24	1	0
3.25 to 3.34	0	0
3.35 to 3.44	0	0
3.45 to 3.54	1	0
3.55 to 3.64	0	0
Total	400	400

The class of ratio values into which the greatest number of conidia fell for both strains was 1.25 to 2.14. The average ratio of length to width was 1.68 for both strains. While this ratio is somewhat larger than the measurements showed in the previous work (7), it is not of a sufficient variance, when all other morphological characters are taken into consideration, to warrant making a new species for both strains. Rosenbaum (*8*) in his description of *Phytophthora faberi* Maubl. gives a mean ratio of 1.47 and this figure was more clearly obtained by the writer in his previous measurements of the coconut strain (7). Graphic representations of the arrangements in classes of the ratio of the length to the width of conidia, showing the limits of variation for both strains, are given in figures 3 and 4.

Germination of the conidia.—Germination takes place by the production of either germ tubes or swarm spores. Every conidium is potentially a sporangium; its method of germination is influenced greatly by its environment. Germination by germ tubes is by far the commoner method under cultural conditions. From 1 to 5 germ tubes may be produced; these apparently may develop from any part of the surface of the conidium (Pl. 10, C). Both strains, under favorable conditions, will produce an abundance of zoospores. These conditions are not clearly understood. Attempts to produce zoospores from spore suspension of cornmeal cultures in sterilized and tap water by placing vials containing 5 cc. of these suspensions at various temperatures in Altmann regulated incubators from 4° up to 30° C., failed to produce zoospores. Excellent direct germination was obtained. Frequently old oatmeal agar cultures, from which the surface mycelium had been removed and then new growth allowed to start, would produce zoospores by the addition of water. Swarm spores were also obtained in Van Tieghem cells by growing the organisms on very dilute agar or in hanging drops of water placed on sterile cover slips. Just before formation there appeared a rearrangement of the protoplasmic granules. The swarm spores were then produced within the sporangium (Pl. 11, A and B). The end of the papilla finally broke off or was dissolved and the spores escaped. No vesicle formation has been observed. The spores approach the opening and escape one by one. When produced on dilute agar the zoospores oozed out of the sporangium and remained in a mass at the mouth. Here they soon germinated by sending out germ tubes (Pl. 11, C and D). Frequently zoospores did not escape from the sporangium and then germination took place within the sporangium (Pl. 11, C and D).

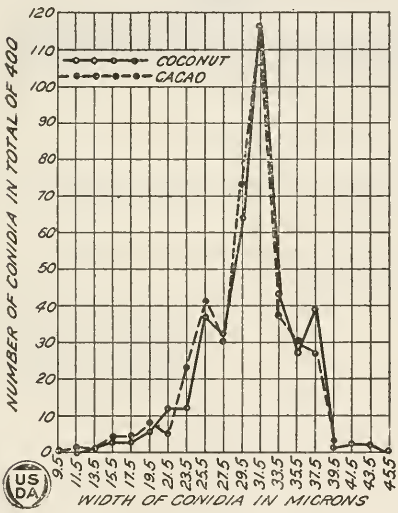

Fig. 2.—Graph showing the variation in width of conidia.

The process of emerging from the sporangium in water cultures is extremely interesting. After the dissolution of the tip of the papilla an active movement of the developed zoospores within takes place. As

soon as a zoospore finds the opening it comes to rest and gradually oozes out, becoming much elongated in the process and constricted in the middle and bulging out at both ends, due to the smallness of the opening (Pl. 11, A and B). After the zoospore passes through, it comes to rest for a portion of a second and then swims off actively. While one zoospore is passing out, the rest within swim about against the wall until the opening is again free, when one by one they will escape in the same manner. The zoospores are granular, slightly greenish, and have distinct nuclei and one or two vacuoles. From a side view they are kidney shaped, and when observed from the end a groove is seen on one side from tip to tip. The swarm spores swim about for a time by means of two flagella. Just

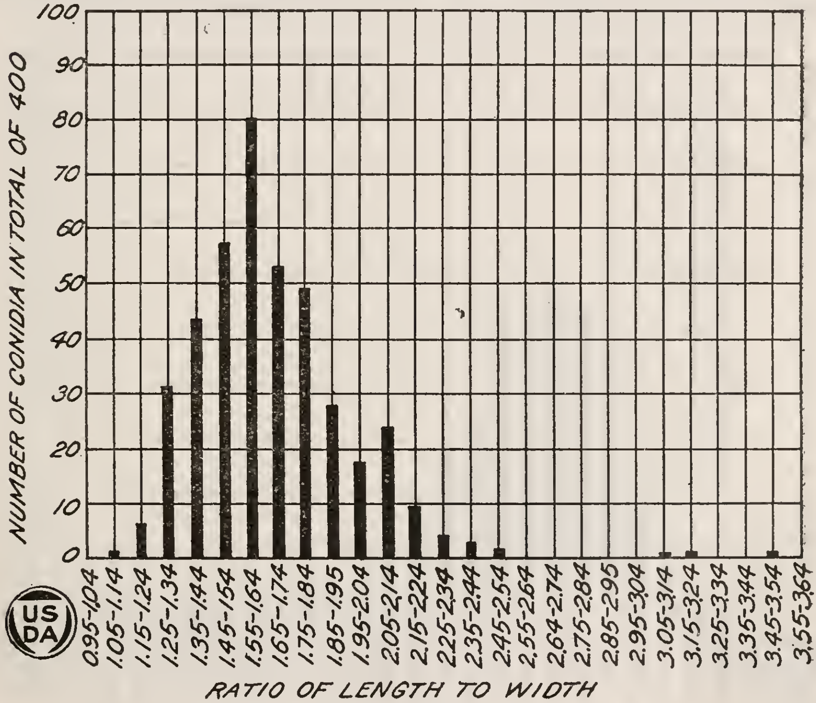

Fig. 3.—Arrangement in classes of the ratios of the length to the width of conidia (coconut), showing the limits of variation.

before coming to a permanent rest the spores become sluggish, settle down for a time, then start off, settle down again, and swim away once more, and finally settle down again definitely. No distinct spore wall was noted on the motile spores, but as soon as they came to a permanent rest a wall could be distinguished, which was especially clear when germination started (Pl. 11, A and B). In the rest stage the spores became spherical, in some cases with a depression on one side, took on a more greenish tinge, became more granular, enlarged somewhat, and then put out germ tubes which grew into a mycelium (Pl. 11, A and B). In 25 minutes the germ tube of one zoospore on potato dextrose agar was observed to double in length. Frequently in hanging drop slides of water and dilute agar all the zoospores were produced from secondary sporangia that arose from the original spores placed in the drop or on the agar.

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The original spore in these cases first germinated by the production of a germ tube that grew into a short mycelium, which in turn became a sporangiophore producing a group of sporangia. Some of the latter would then produce zoospores.

CHLAMYDOSPORES

Chlamydospores are produced directly from the mycelium, usually terminally, but sometimes intercalarily (Pl. 12, C and D). They are spherical, granular like the conidia, but with a slightly deeper yellow shade (Pl. 8, E). In pure culture they are produced in great abundance on oatmeal or potato dextrose agar. In the diseased rind of cacao fruits many thick-walled chlamydospores are usually produced (Pl. 8, F).

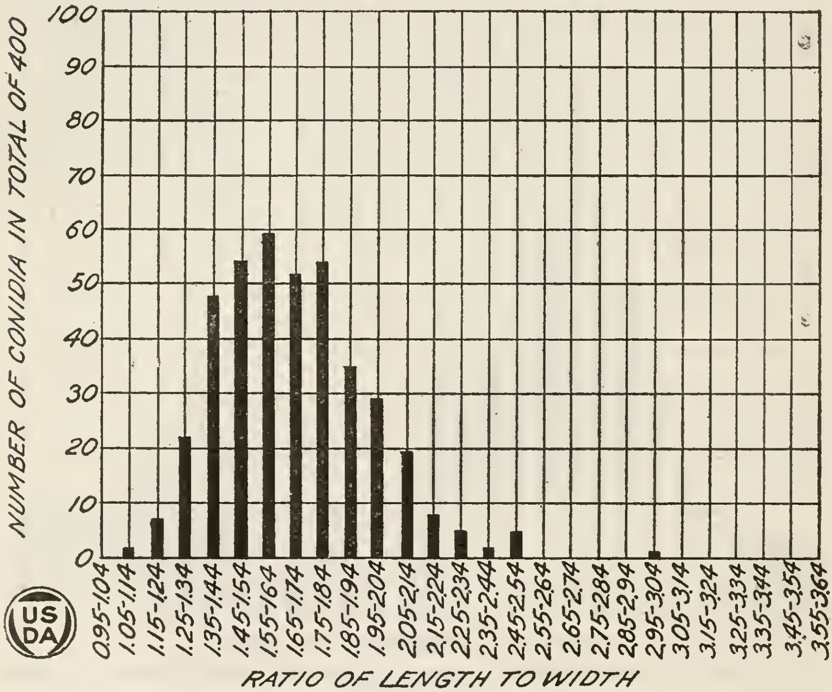

FIG. 4.—Arrangement in classes of the ratios of the length to the width of conidia (cacao), showing the limits of variation.

MEASUREMENTS OF CHLAMYDOSPORES.—The size of the chlamydospores is one of the criteria by which the species of Phytophthora are separated. Four hundred measurements were made of the diameters of the spores from both coconut and cacao strains grown on oatmeal agar. The coconut culture was 9 to 10 days old, and that of cacao was 11 days old. A summary of these measurements, grouped in classes, appears in Table III.

The chlamydospores of the coconut strain varied in diameter from 19.5 to 61.49 μ, the average diameter being 41.62 μ. These measurements were made in May, 1922, of spores grown on oatmeal agar and correspond favorably with those made in 1919 grown on corn meal (7).

For the cacao strain the chlamydospores varied in diameter from 17.5 to 53.49 μ, the average diameter being 41.06 μ.

TABLE III.—*Summary of measurements of chlamydospores from coconut and cacao*

Class (in microns).	Number of chlamydospores in each class from—	
	Coconut.	Cacao.
15.5 to 17.49	0	0
17.5 to 19.49	0	1
19.5 to 21.49	4	0
21.5 to 23.49	1	0
23.5 to 25.49	1	1
25.5 to 27.49	3	3
27.5 to 29.49	5	3
29.5 to 31.49	1	4
31.5 to 33.49	10	7
33.5 to 35.49	6	8
35.5 to 37.49	16	25
37.5 to 39.49	70	69
39.5 to 41.49	49	69
41.5 to 43.49	69	81
43.5 to 45.49	91	99
45.5 to 47.49	37	17
47.5 to 49.49	22	8
49.5 to 51.49	12	3
51.5 to 53.49	1	2
53.5 to 55.49	1	0
55.5 to 57.49	0	0
57.5 to 59.49	0	0
59.5 to 61.49	1	0
61.5 to 63.49	0	0
Total	400	400

The measurements, therefore, for both strains are so nearly identical that from this standpoint both fall under the same species. A graphic representation of the measurements for both strains is given in figure 5.

GERMINATION OF CHLAMYDOSPORES.—In Van Tieghem cells, prepared with hanging drops of distilled water, corn meal extract, potato dextrose agar, or pure agar, direct germination took place within 24 hours. From 1 to 12 germ tubes may arise from one spore.

SEXUAL BODIES

No sexual bodies have been observed in diseased portions of coconut or cacao trees or in pure cultures of either of these strains. The absence of antheridia places these strains in the Faberi group, in accordance with Rosenbaum (*8*), "which embraces the forms in which the antheridia are entirely absent or in which the relation of the antheridium to the oogonium is unknown."

TAXONOMY

According to the tentative table offered for the separation of species of Phytophthora and devised by Rosenbaum (*8*), both strains fall into the Faberi group and in the species *Phytophthora faberi* Maubl. The discrepancy in the case of the ratio of the length to the width of the conidia, alone, does not warrant the creation of a new species for either strain, especially in the present state of the Phytophthora group. The descriptions and measurements of both strains also correspond to those of the original description of the species by Maublanc (*4, p. 314–324*).

DISCUSSION

RELATIONSHIP

The two strains of Phytophthora are, therefore, to be considered as identical. Both behave alike in infection experiments. The physiological characteristics of each from a standpoint of growth on various media, relation to light, relation to temperature, and relation to acidity and alkalinity are the same. The morphological characteristics are also so nearly alike that no real distinction can be made between them. Both strains of the fungus correspond closely to the Faberi group and to the species *Phytophthora faberi* Maubl. as determined by Rosenbaum (*8*). Antheridia are entirely unknown. The papillae of the conidia are raised and very prominent. The measurements of conidia and chlamydospores closely approximate those for this species. There is a slight variation in the ratio of the length to the width of conidia. This difference, when taken into consideration with all the other points of similarity, is not sufficient to warrant the creation of a new species. Rosenbaum (*8*) gives 1.47 as the mean ratio of length to width of conidia of *Phytophthora faberi* Maubl. The ratio obtained in the last comparative measurements for both strains on oatmeal agar was 1.60. Measurements made in 1919 (7) of the coconut strain grown on corn meal gave a mean ratio of 1.51, being more nearly that as given by Rosenbaum. The slight change in the form of the spores measured in 1919 and 1922 can not be accounted for unless it be due to a difference in age and a change in the medium used.

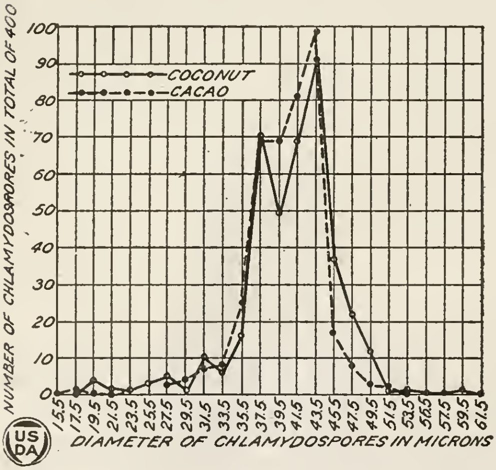

FIG. 5.—Graph showing the variation in the diameter of chlamydospores.

The original description of *Phytophthora faberi* Maubl. as given by Maublanc (*4*) in 1909 on cacao (*Theobroma cacao*) corresponds also very closely to that of the two strains studied by the writer.

In 1907 Butler (*2*) described a new species of a Phycomycete occurring on various hosts, among which was the coconut (*Cocos nucifera*), under the name of *Pythium palmivorum*. Later in 1918 (*3*), the genus was transferred to that of Phytophthora without redescription. In 1907 Ashby (*1*), in an article on two diseases of coconut palm in Jamaica, ascribed the cause of the budrot to *Phytophthora palmivorum* Butl. after having submitted a culture to Butler for comparison. A culture of the fungus from Jamaica (Ashby, subculture of 31–8–20), obtained from Mr. A. Sharples, mycologist of the Federated Malay States, appears to be very much like that of the Phytophthora on coconut and cacao from the Philippines. No accurate comparisons, however, were made. The Phytophthora from the Philippines in all probability is different from that in India, as originally described under *Pythium*

palmivorum, and then later referred to *Phytophthora palmivorum*, as the latter was reported to produce antheridia and oogonia. The development of these organs was not, however, determined with certainty. Ashby (*1*) also observed antheridia and oogonia in his strain from coconut.

In addition to these two hosts, Phytophthoras have been obtained from the following various plants in the Philippines: Two apparently different strains causing the fruitrot and blight of eggplant (*Solanum melongena*); a strain causing the blight of roselle seedlings (*Hibiscus sabdariffa*); a strain causing the blight of citrus seedlings and buds of budded plants (*Citrus* spp.); a strain producing the rot of abaca suckers (*Musa textilis*); and a strain causing the blight of Hevea rubber (*Hevea brasiliensis*). From a general observation some of these strains appear to be identical with those described from coconut and cacao. However, until a complete comparative test is made, no definite statement as to their identity can be given. The determination of the relationship of these various strains is of great importance in a study of tropical diseases of plants.

SIGNIFICANCE OF SIMILARITY OF BOTH STRAINS

The discovery of the similarity of the strains from coconut and cacao is of extreme consequence from a control standpoint. In certain coconut sections it is a common practice to interplant with cacao. As the latter tree is practically always infested with the Phytophthora, this custom will have to be discontinued. It seems highly probable that a number of the other strains listed above may be identical with those on coconut and cacao. A study of these would prove to be of great interest and would bring out new means of combating the diseases.

SUMMARY

Coconut culture is one of the chief industries of the Philippine Islands. Millions of trees have been planted on the various islands of the group. The infectious type of budrot has made its appearance in certain sections, primarily in the extensive coconut regions in Laguna, Batangas, and Tayabas Provinces on the island of Luzon. Here sporadic epidemics of the disease have been authentically reported since 1908, and thousands of dollars of loss has occurred since that time.

Various organisms, including bacteria and fungi, have been considered responsible for the disease. In the Philippine Islands there are apparently two types of budrot, the really infectious type caused by a Phytophthora, and a secondary type following some injury such as beetle injury and caused primarily by the invasion of bacteria in the weakened tissue.

The culture of cacao in the Philippine Islands, while not extensive, can be considered as one of the secondary industries. The manner in which the trees are grown by the average farmer subjects the trees to severe fungus attacks. In certain sections frequently one-half of the cacao crop is destroyed. The canker of the branches and trunk, and the rot of the pods are the chief troubles, and they are caused by a Phytophthora. Cacao trees are often interplanted with coconut trees, offering a ready means of transfer of a fungus from one plant to the other.

A careful study of the cross infection possibilities and the physiological and morphological characteristics of the fungus strains from the

coconut and cacao has shown that the coconut budrot and the blackrot and canker of cacao are caused by the same species of Phycomycete, *Phytophthora faberi* Maubl. The discovery of the identity of these two strains is of utmost importance from a control standpoint. Coconut and cacao trees should not be interplanted, since the fungus can be readily transmitted from one host to the other.

Similar types of coconut budrot in India and Jamaica have been shown by the investigators in those countries to be due to a Phytophthora. The similarity of the strains on coconut and cacao has been determined only in the Philippine Islands. A comparative study of the Phytophthoras from India and Jamaica would be of interest.

The presence of other strains of Phytophthora on important economic plants in the Philippine Islands, with the possibilities of cross inoculation between these, indicates that some may be identical with the strains on coconut and cacao.

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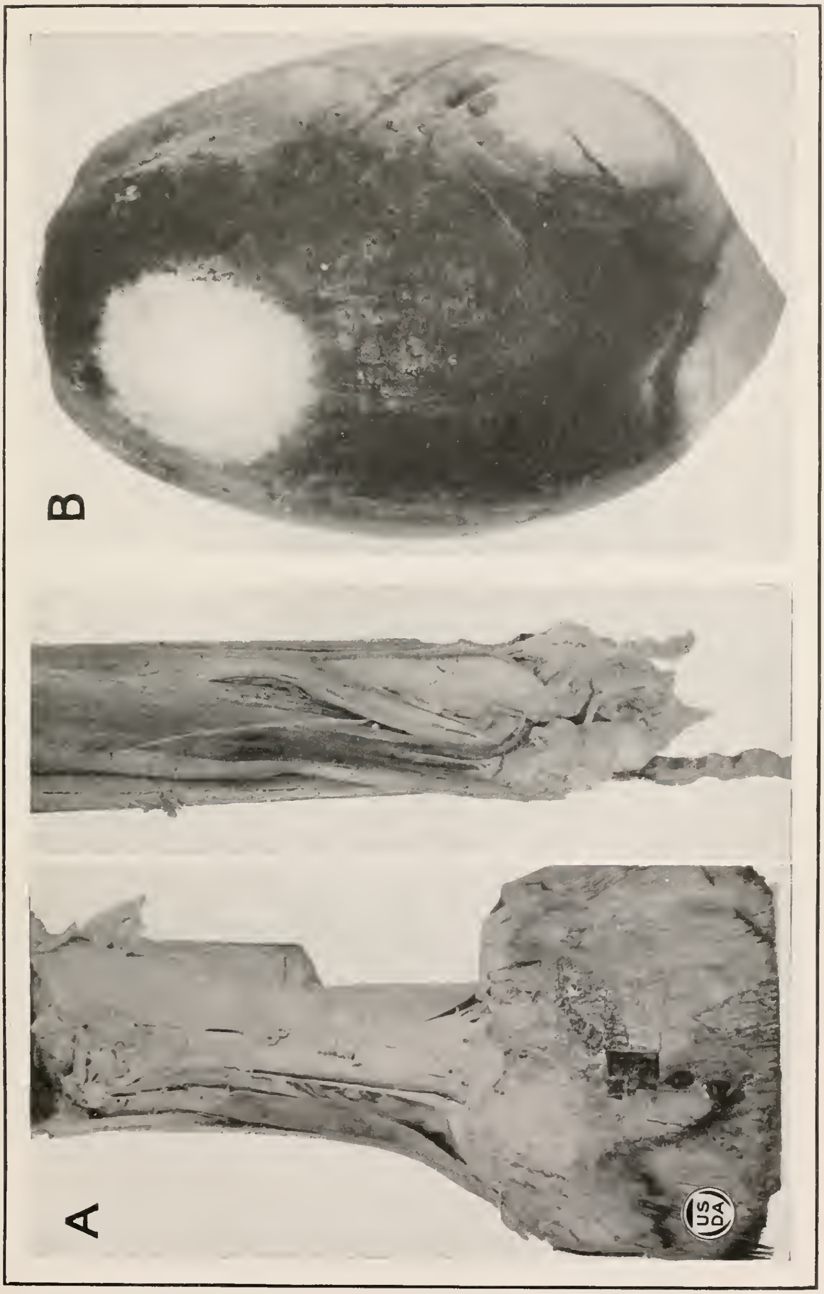
PLATE 1

A.—Typical field case of coconut budrot showing infection of region about growing point and in bud. From this specimen, obtained on March 11, 1919, at Lilio, Laguna Province, P. I., the pure culture of the fungus strain used for the coconut experiments was obtained. The photomicrographs (Pl. 8) showing the mycelium and haustoria in the tissue were made from sections obtained from this particular specimen.

B.—Early stages in rot of papaya (*Carica papaya*) produced by inoculation with Phytophthora strain from coconut. A similar infection is produced by the cacao strain.



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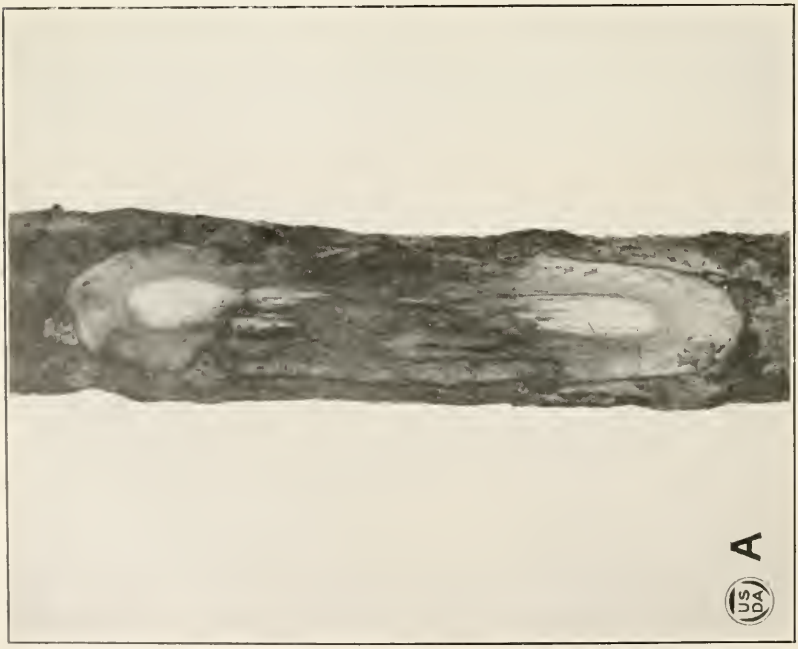

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PLATE 2

Canker of cacao branches and blackrot of cacao pods.

A.—Canker of cacao branches produced by inoculation with Phytophthora strain from cacao.

B. Blackrot of cacao pod produced by inoculation with Phytophthora strain from cacao.

PLATE 3

Typical cases of coconut budrot produced by inoculation with Phytophthora strains from coconut and cacao.

A.—Budrot produced in healthy, vigorously growing coconut tree by merely pouring a zoospore suspension in rain water of the coconut strain between the growing leaves of the bud. Bud killed in 15 to 16 months after inoculation.

B.—Budrot produced in a disease-free coconut tree by inoculation with a zoospore suspension of the cacao strain. The tree was also infested with beetles, as indicated by the leaves with triangular shaped portions cut out of the leafleats. Bud killed 2 to 3 months after inoculation. The rapid production of the rot in this case was undoubtedly due to the beetle injury to the leaves, and also to the fact that the tree was not in a vigorous condition of growth.

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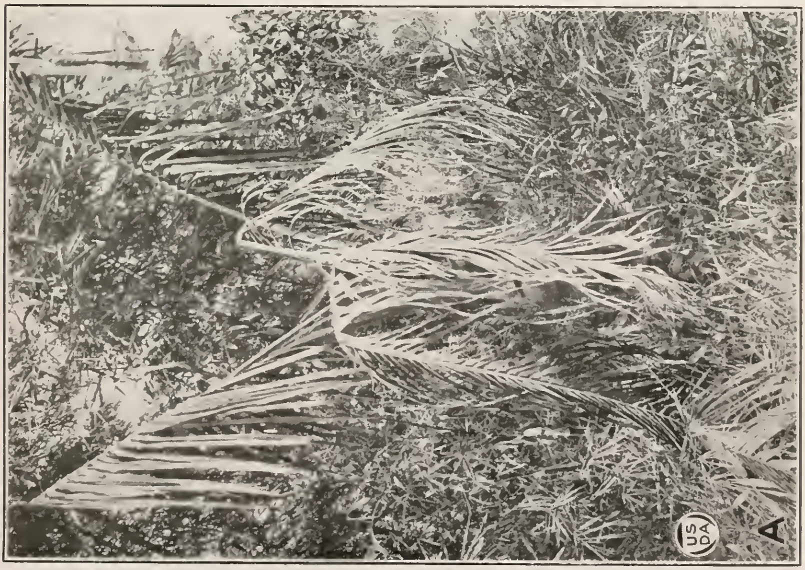

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PLATE 4

Coconut budrot produced by inoculation of healthy tree with Phytophthora strain from coconut.

A.—Longitudinal section of diseased tree shown in Plate 3, A, showing typical rot of region about growing point and in lower part of bud.

B.—Diseased tree, on right, and healthy control tree, on left, 15 to 16 months after inoculation. At time of inoculation both trees were of approximately the same age and height. The stunting of the infected tree is clearly shown.

PLATE 5

Coconut seedlings inoculated with Phytophthora strain from coconut.
A.—Inoculated tree and control. Stab method of inoculation employed.
B.—Typical and severe rot produced in seedling, using stab method of inoculation.


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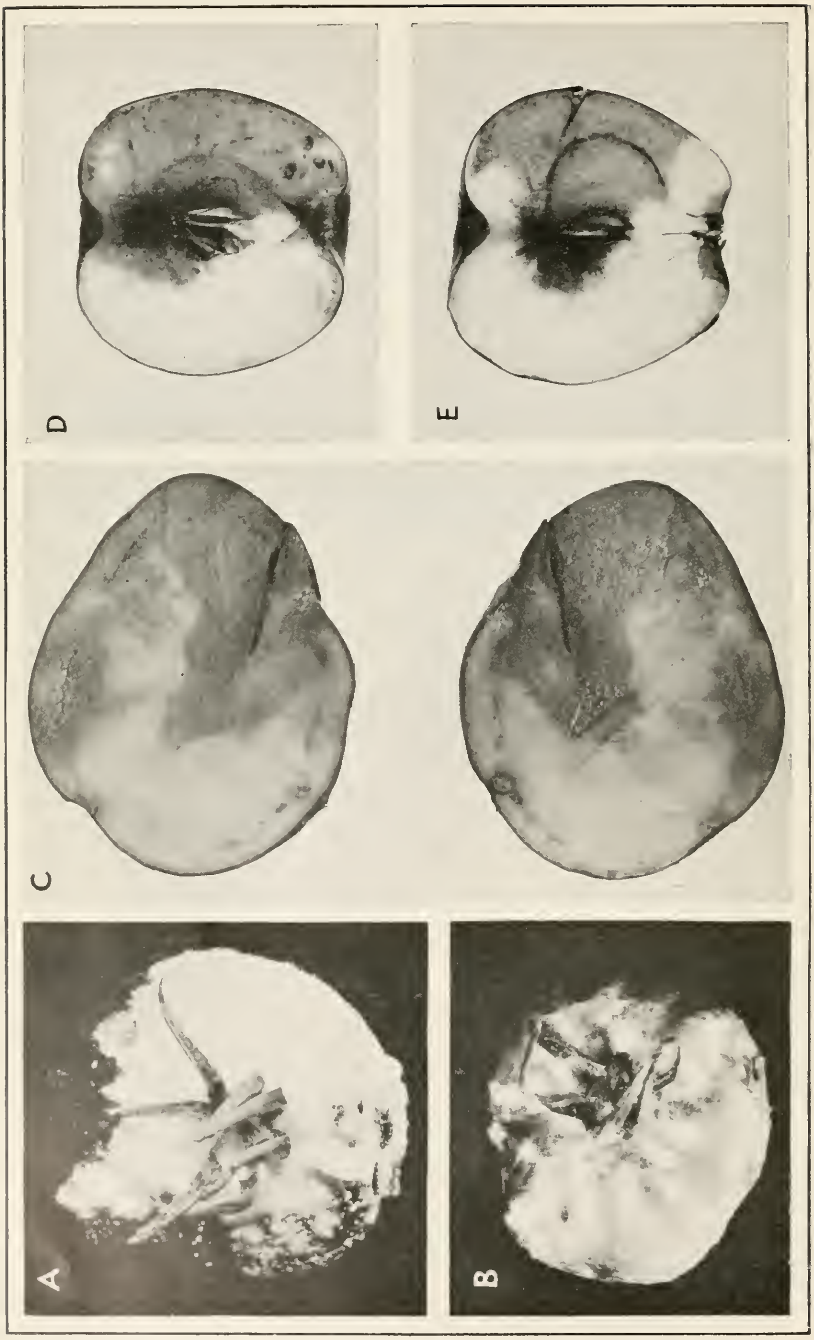
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PLATE 6

Rot of tomato, potato, and apple produced by inoculation with both strains of Phytophthora.

A.—Rot of ripe tomato produced by inoculation with Phytophthora strain from cacao.

B.—Rot of ripe tomato produced by inoculation with Phytophthora strain from coconut.

C.—Rot of potato produced by inoculation with Phytophthora strain from coconut. Rotted area pinkish in color. A similar rot is produced by Phytophthora strain from cacao.

D.—Rot of Ben Davis apple produced by inoculation with Phytophthora strain from cacao.

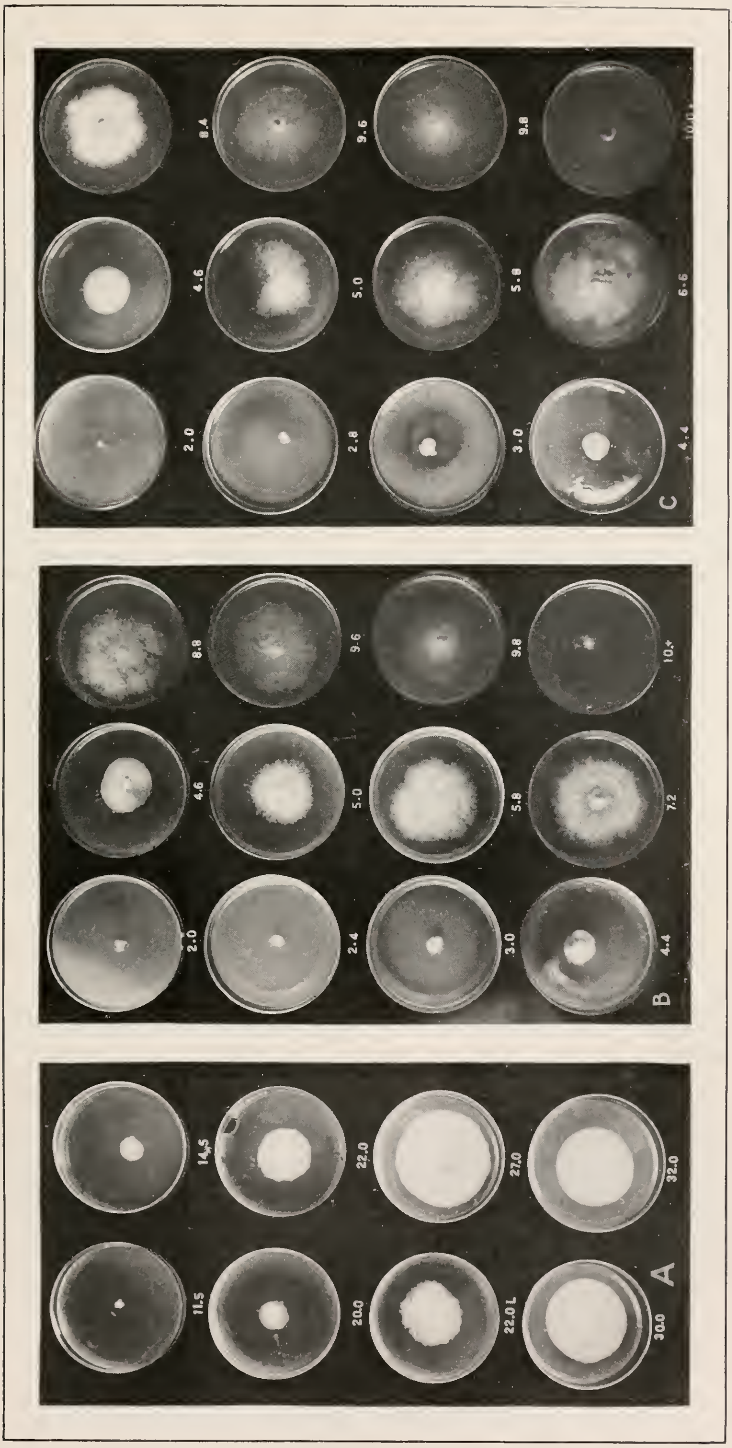
E.—Rot of Ben Davis apple produced by inoculation with Phytophthora strain from coconut.

PLATE 7

A.—Growth of coconut strains on potato dextrose agar made in Altman incubators at various temperatures. The effect of light and darkness is clearly shown in the two plates grown at the same temperature: 22.0 in the dark; 22.0L, in the light. A fluffy, granular growth due to sporulation is produced in the light.

B.—Growth on potato dextrose agar at room temperature of the Phytophthora strain from coconut under various hydrogen-ion concentrations.

C.—Growth on potato dextrose agar at room temperature of the Phytophthora strain from cacao under various hydrogen-ion concentrations.



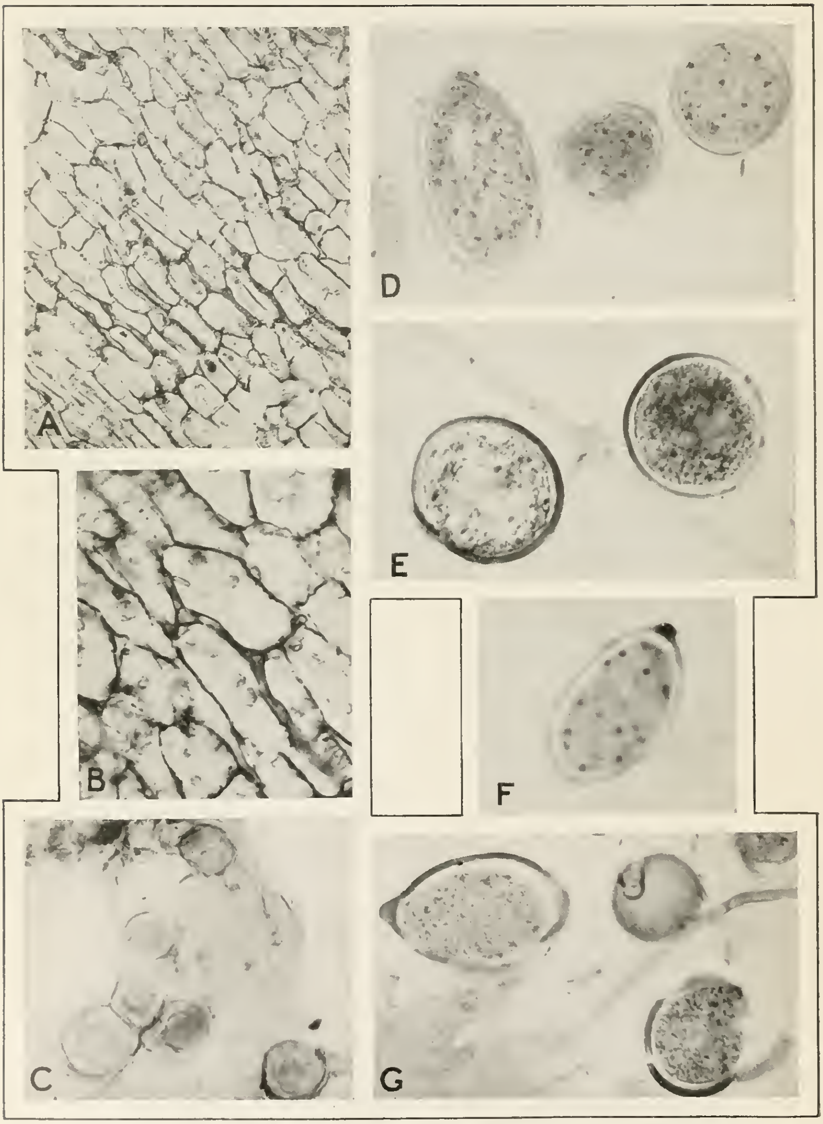

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PLATE 8

Photomicrographs of diseased tissues of coconut and cacao:

A.—Section from diseased tissue near growing point of field case of coconut budrot, showing intercellular, nonseptate mycelium. Approximately, × 200. Acid fuchsin and light-green stain.

B.—Section from diseased tissue near growing point of field case of coconut budrot, showing fingerlike haustoria penetrating host cells. Approximately, × 750. Acid fuchsin and light-green stain.

C.—Free-hand section of rind of diseased cacao pod, showing chlamydospores. Approximately, × 250. Acid fuchsin and light-green stain.

Photomicrographs of conidia and chlamydospores of both strains of Phytophthora:

D.—Conidia of Phytophthora strain from coconut, showing presence of many nuclei. Sectioned material from pure culture of fungus on oatmeal agar. Approximately, × 750. Triple stain.

E.—Chlamydospores of Phytophthora strain from cacao. Sectioned material from pure culture on oatmeal agar. Approximately, × 750. Triple stain.

F.—Conidium of Phytophthora strain from coconut, showing papilla stained deeper than wall of spore, and numerous nuclei. Sectioned material from pure culture on oatmeal agar. Approximately, × 750. Triple stain.

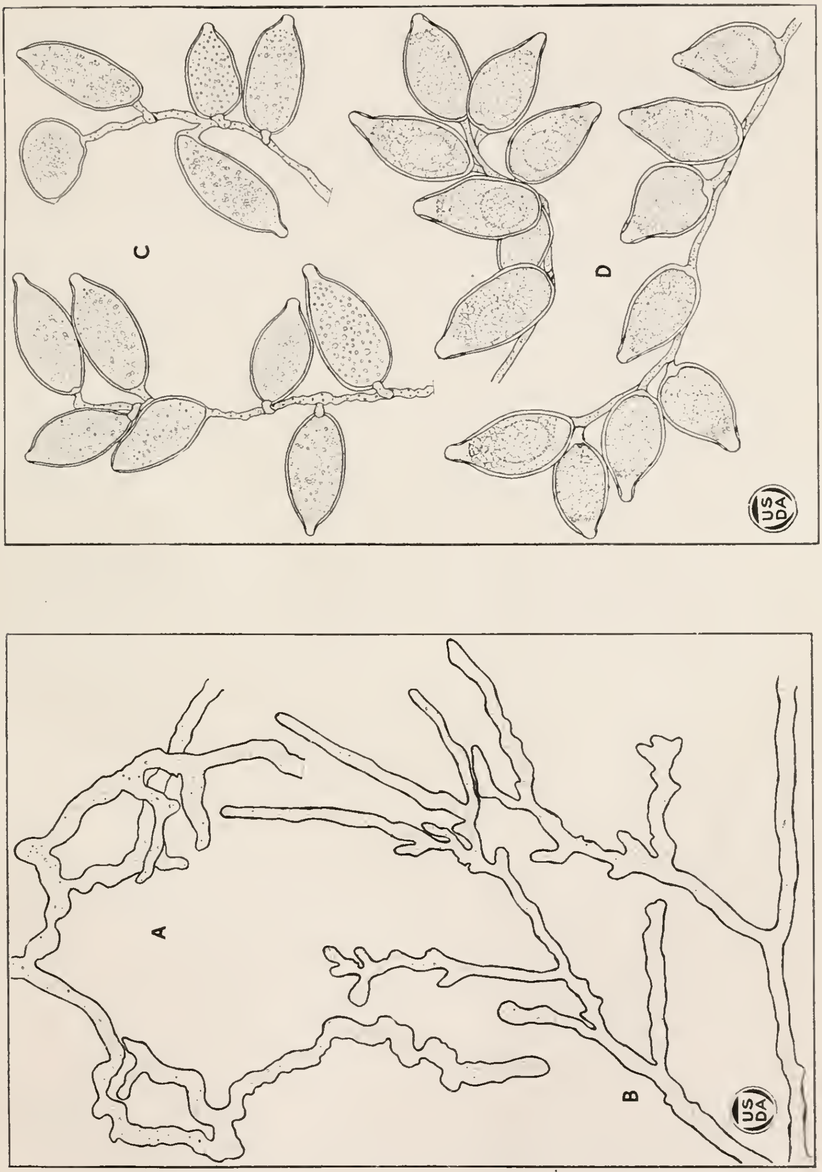
G.—Conidia of Phytophthora strain from cacao showing presence of many nuclei. Sectioned material from pure culture of fungus on oatmeal agar. Approximately, × 750. Triple stain.

PLATE 9

Mycelium, conidiophores, and conidia of both strains of Phytophthora.

A, B.—Submerged mycelium of both strains of Phytophthora grown on potato agar. × 400. A, coconut. B, cacao.

C, D.—Conidiophores and conidia of both strains of Phytophthora. × 400. C, coconut. D, cacao.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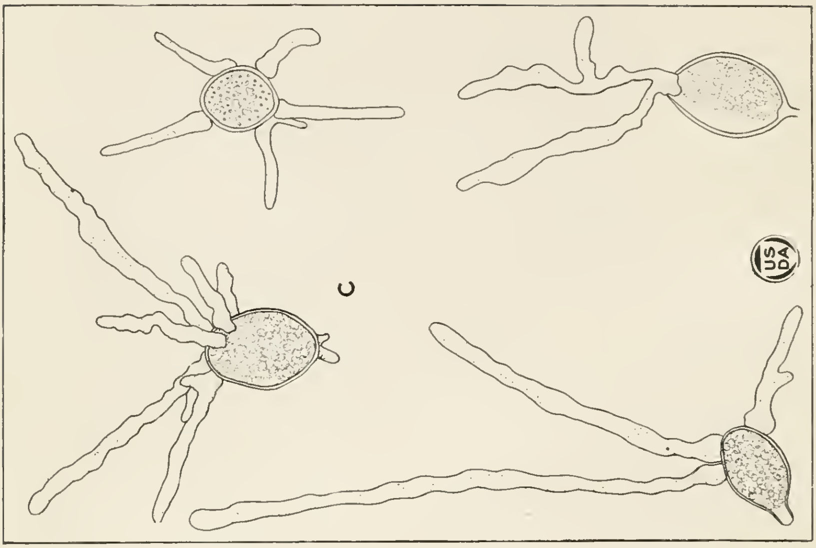
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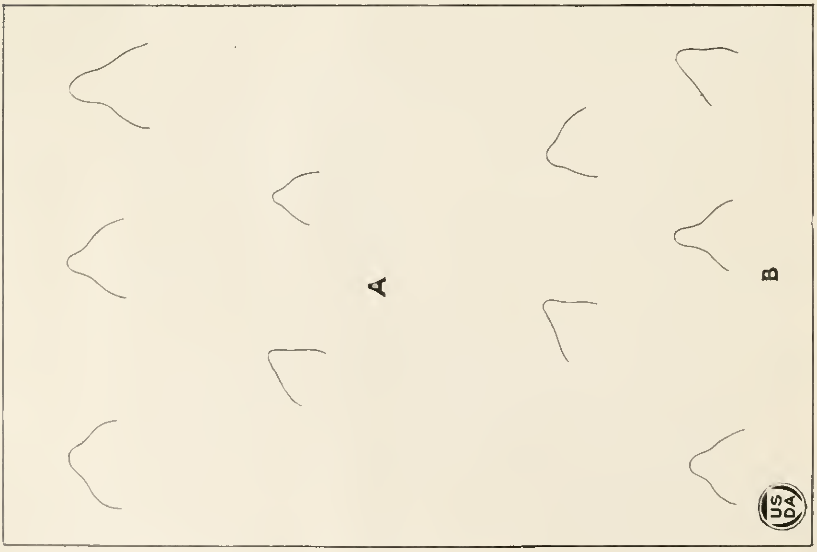
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PLATE 10

A, B.—Prominent, raised, terminal papillae from conidia of both strains of Phytophthora grown on potato agar. × 400. A, coconut. B, cacao.

C.—Direct germination of conidia of the Phytophthora strain from coconut showing the production of from few to many germ tubes. × 400.

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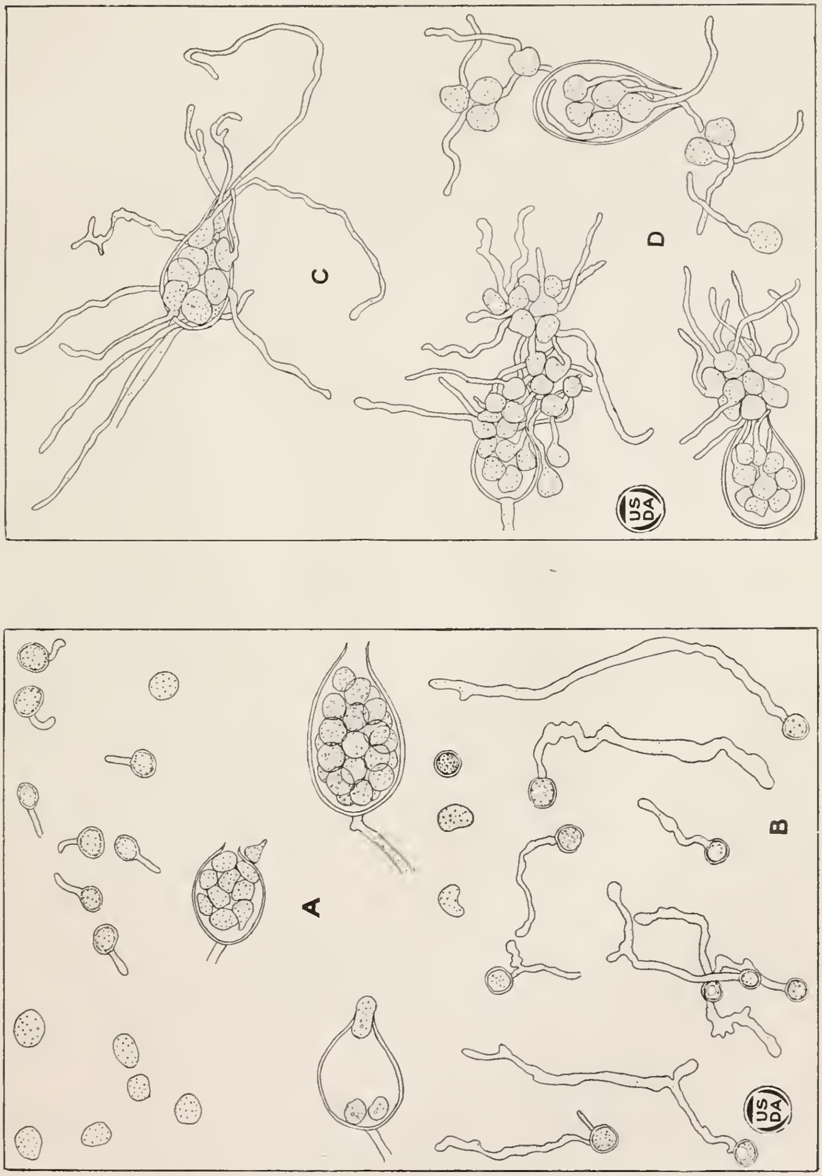
PLATE 11

Zoospore production and germination in various stages of development of both strain of Phytophthora.

A, B.—Zoospore production showing various stages of development and germination. × 400. A, coconut. B, cacao.

C, D.—Zoospore germination in place within the sporangium and at the mouth of the sporangium. × 400. Produced in Van Tieghem cells on dilute agar. C, coconut. B, cacao.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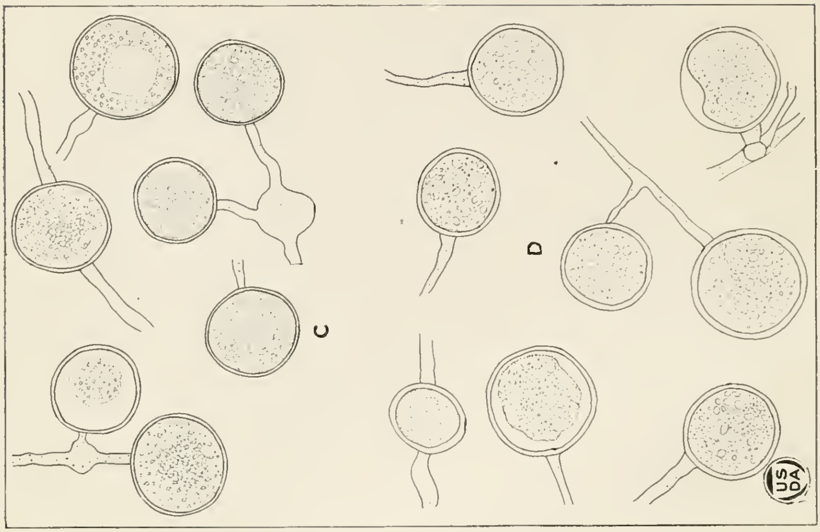
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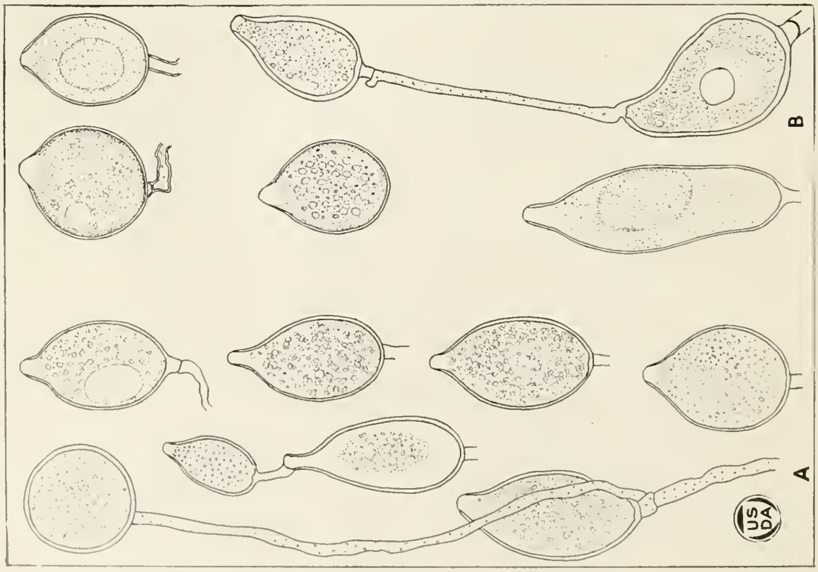
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PLATE 12

Various types of conidia and chlamydospores of both strains of Phytophthora.

A, B.—Conidia of various forms of both strains of Phytophthora. × 400. A, coconut. B, cacao.

C, D.—Chlamydospores of various sizes of both strains of Phytophthora. × 400. C, coconut. D, cacao.

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QUANTITATIVE VARIATION OF GOSSYPOL AND ITS RELATION TO THE OIL CONTENT OF COTTONSEED[1]

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INTRODUCTION

In connection with the food conservation problem which arose during the World War, the Bureau of Chemistry was asked to supply information on the suitability of cottonseed press cake for human consumption.[2] As the statements in the literature on the nature of poisoning by cottonseed were conflicting, a reinvestigation of the question was necessary. The investigation resolved itself into several distinct, though closely related, studies. While all of these studies were carried on simultaneously, each one will be reported separately, beginning with the one here discussed.[3]

For a long time some feeders have believed that different lots of cottonseed meal have different degrees of toxicity. [5] It is highly probable that there is a sound basis for this belief on the part of practical men. Crawford (*6*), who stated that the toxic agent in cottonseed is pyrophosphoric acid, regarded some of these differences, at least, as varietal characteristics of the different seed. This would be consistent with the belief very generally held that cottonseed poisoning is more frequent in some regions than in others, since the varieties cultivated differ from region to region. At any rate, it seems to be a fact that a variation in toxicity is associated with variations in the place and crop year of production, although the writers have been unable to find in the literature satisfactory data on which a comparison of poisoning by cottonseed produced in different regions or from the crops of different years in the same region could be based.

That the composition of many seeds varies from region to region and from crop year to crop year has been established beyond doubt. In the case of wheat these variations are common knowledge. Piper and Morse (*8*) have observed that certain regions of the South produce soybeans with higher oil content than others. Thompson and Bailey (*11*) found that differences in the oil content of different varieties of peanuts grown under the same conditions and in the same locality were not

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[1] Accepted for publication June 23, 1923. This is the first paper of a series dealing with cottonseed poisoning.

[2] Fraps (*7*) [4] recommends cottonseed meal, in limited quantities, for human consumption. He does not deny, however, the deleterious action of this meal on domesticated animals.

[3] The preliminary report on this work was read before the American Chemical Society, April 7–11, 1919. (See: ALSBERG, C. L., SCHWARTZE E. W., and WHERRY, E. T. THE OCCURRENCE OF GOSSYPOL IN DIFFERENT VARIETIES OF COTTONSEED. (Title.) *In* Science, v. 49, p. 573. 1919.)

[4] Reference is made by number (italic) to "Literature cited," p. 295.

[5] Personal communication from C. T. Dowell, Stillwater, Okla.

pronounced. Similar facts have been established for cottonseed by Bidwell,[6] who has shown that cottonseed from the southwestern United States, on the average, is low in oil and high in protein, while seed from the Atlantic and Pacific coasts has a higher oil and lower protein content in the order named. While Bidwell's generalization is undoubtedly justified, exceptions must be anticipated, for over such large territories climatic and soil conditions must be exceptional here and there. This being so, exceptions will be encountered, if any reliance can be placed upon the work of Bain and Anders as reported by Cook (*5*), who writes as follows:

> The fluctuations induced by conditions of growth or associated with various degrees of maturity attained by the seeds were so large as to conceal inherent differences in individual plants or progenies.

Hence the finding by Rast (*9*) of great variations in composition of Georgia cottonseed is not astonishing nor to be regarded as invalidating Bidwell's generalization.

If it be true that cottonseed varies greatly in composition and toxicity in different parts of the country and perhaps also in different crop years, then the toxic factor in cottonseed, whatever it may be, might well vary correspondingly. If Withers and Carruth (*12*, *13*) are right in attributing the toxicity of cottonseed to the presence in it of a phenolic substance named by Carruth "gossypol," then the gossypol content of different samples of cottonseed should vary as the toxicity varies. Apparently Carruth (*3*), recognizing that gossypol occurs in the so-called "gland dots" or "resin glands" in cottonseed, the distribution of which has been studied by Stanford and Viehoever (*10*), does not believe the gossypol content of cottonseed to vary greatly. He states that "since all varieties of seed seem to have approximately the same number of glands, it would appear that gossypol does not vary to a greater extent than the oil or protein content." This statement, however, is not based on experimental evidence. Hence it is just as reasonable to assume that, even if the number of glands in different cottonseeds were the same, the glands might vary both in size and in gossypol content. Carruth (*3*) found gossypol in cotton-root bark, where, according to the histological studies of Stanford and Viehoever (*10*), there are also internal glands. Carruth (*3*) also attributed the differences of toxicity to variations in the method of manufacture of the press cake. However, this suggestion, while it may apply in some cases, does not explain the infrequency of poisoning in certain regions where the method of manufacture of the meal is generally the same as that in regions where poisoning is prevalent.

There is, then, very definite evidence that the composition of cottonseed varies widely and no evidence inconsistent with the possibility that the gossypol content may vary correspondingly. To determine whether or not the gossypol content of cottonseed varies and to determine whether there is any correlation between the gossypol, the protein, and the oil content is the purpose of the present paper. A study of the oil and protein content was included in the investigation, so that three criteria instead of one might be available for checking the results. The protein and oil analyses could be used to determine whether or not the sample was representative of the region from which the seed came and also to rule out "sports" or atypical seed. Consideration of the oil and protein content made it unnecessary to consider in detail the various factors affecting the seed.

[6] Personal communication from G. L. Bidwell, Bureau of Chemistry.

This study, then, deals solely with the maximum variation of the gossypol content of cottonseed and the correlation of the gossypol content with the oil and protein content of the seed. No consideration is given to the biological factors that might cause the gossypol content to vary. No consideration is given to methods of selection, breeding, or cultivation that might lead to the production of seed of low gossypol content.

SOURCES OF SEED EXAMINED

Samples of most of the standard varieties and some of the same variety from different localities were secured. Most of the seed examined was obtained through the offices of O. F. Cook and R. A. Oakley, of the Bureau of Plant Industry, United States Department of Agriculture, which maintains an inspection of plantations on which its seeds are grown. Therefore, there can be no doubt as to the authenticity of the varieties of the seed furnished. A few samples were obtained directly from planters, experiment stations, or dealers.

METHODS OF EXAMINATION

Only the kernels or meats of the seeds were examined. Usually a very small quantity of hull and lint, which could not be removed from the ground material by sifting, was present. Gossypol was isolated as the "acetate" by the methods of Carruth (*3*) from all varieties of seed used. All samples of gossypol "acetate" isolated were examined crystallographically. Quantitative analysis was made according to the writers' modification of the aniline method of Carruth (*4*). The fat and moisture determinations upon the samples of material analyzed for gossypol were made in the Cattle Food Laboratory, and the nitrogen determinations by the Kjeldahl method were made in the Nitrogen Section of the Bureau of Chemistry.

MODIFIED ANILINE METHOD

Place 75 gm. of practically hull-free finely ground cottonseed meats in a Soxhlet extraction thimble. Extract with ether until the thimble which stands in the ether overnight imparts to it no significant yellow color. Evaporate the ether completely and transfer the extract to a beaker, using petroleum ether to work it over with. If necessary, filter it. Use 8 to 10 times as much petroleum ether as the volume of the extract.

After standing overnight, a very small quantity of a fine flocculent precipitate appears. This is not gossypol, for while gossypol is not soluble in petroleum ether alone, it remains in the oil-petroleum-ether mixture. Filter off this petroleum-ether-insoluble material extracted by the ether and wash the precipitate with petroleum ether. Wash the precipitate and the filter paper with ether. Filter this ether solution and evaporate almost completely. Mix the residuum with petroleum ether in order to hold in solution the last traces of oil and petroleum-ether-soluble material. Then filter and combine the filtrate with the main petroleum-ether solution. Add 1 cc. of aniline and dissolve it in the solution by shaking. Unless dissolved, the gossypolaniline compound comes out in clusters around the small drops of aniline.

In from 3 to 7 days later filter the precipitate of aniline-gossypol compound through a tared Gooch crucible and wash it several times with

petroleum ether. Rub off the material adhering to the precipitating flask with a rubber-tipped glass rod or dissolve it in ether, from which it may be precipitated by almost completely evaporating the ether, adding petroleum ether, and reevaporating to a small volume. Then pour it carefully into the Gooch crucible. Bring the Gooch crucible and its contents to constant weight at 100° C. by hourly heatings. This precipitate is slightly hygroscopic. Carry on the heating no longer than is necessary. If filter paper has been used in the Gooch crucible, the precipitate can be removed without admixture of foreign substances.

Preserve the filtrate containing the petroleum-ether-aniline mixture and transfer it to an Erlenmeyer flask. Stopper the flask and let it stand in the cold for from seven days to one month, to ascertain whether all the gossypol has come down. If gossypol appears, allow the petroleum ether to evaporate partly and let stand again. Corrections based on these subsequent precipitates may be made.

APPLICATION OF MODIFIED ANILINE METHOD

Table I shows the gossypol content of several varieties of cottonseed determined by the aniline method of Carruth (*4*) and also by the qualitative "acetate" method (*3*), in which care was taken to make the yields as large as possible. These data show that the recoveries by the "acetate" method are from 16 to 33 per cent lower than those by the aniline method. The approximate agreement of the results obtained by the two procedures lends weight to the assumption that the aniline method gives data representing the gossypol content.

TABLE I.—*Gossypol obtained by the "acetate" and aniline methods*

Variety of cottonseed.	Acetate method.[a]	Aniline method.[b]
	Per cent.	*Per cent.*
Lone Star	0. 27	0. 320
Acala	. 324	. 426
Trice	. 401	. 614
Durango	[c]. 636	. 953
Egyptian	[c]. 610	....
Sea Island	1. 018	....

[a] The acetic acid in gossypol "acetate" is approximately 10 per cent and has been deducted.
[b] A Kjeldahl analysis was made and the nitrogen, calculated as aniline, was deducted from the weight of the precipitate.
[c] Some was lost.

The nitrogen content of the aniline precipitate was from 3.75 to 4.035 per cent. The variation was due, to some extent at least, to the adsorption of free aniline. A lower nitrogen content has been observed and a preparation free from the odor of aniline has been obtained upon recrystallizing several times from chloroform. Usually more nitrogen was present in the more bulky precipitates from the Gooch crucible, owing probably to less favorable conditions for removal of aniline in the heating. The variability in aniline content of the aniline-gossypol compound was noted by Carruth also (*3*). The weight of gossypol was calculated by deducting the weight of the aniline ($N \times 6.64$) from the weight of the precipitate. This correction of the deduction, however, could perhaps

be dispensed with by using the general average, as the increased accuracy obtained by considering variations in nitrogen content is within the limits of error of the method.

The use of petroleum ether as a medium for precipitation facilitated the formation of a more filterable mass, and expedited the separation of the aniline compound. It also accelerated the rate of filtration. During the ether extraction some material other than fat or gossypol was dissolved. This was removed from the petroleum-ether mixture before the aniline was added.

When the crude ether extracts of cottonseed kernels or the mother liquors obtained in the process of recrystallization of gossypol were treated with aniline, the precipitate formed had a dull red color. When this precipitate was recrystallized from chloroform, a few crystals which differed from aniline-gossypol and which could be separated mechanically were usually obtained. When purified gossypol was converted into the aniline compound, crystals of this second type were not obtained. Although the second substance was not studied in detail, it seems probable that it is the aniline compound of the "D-gossypol" of Carruth. The aniline method precipitates not only gossypol, but also gossypollike substances, the quantity of which, however, was relatively small.

Carruth states that the error of his method is less than 10 per cent when 0.5 gm. of gossypol is dissolved in 50 cc. of oil. The results (Table I) by his method, however, are somewhat lower than those subsequently obtained with the same seed by the authors' modification of his procedure. The results of the control analyses for the estimation of known quantities of free gossypol are given in Table II.

TABLE II.—*Estimation of known quantities of gossypol*

Weight of free gossypol taken.	Dissolved in 25 cc. of—	Total weight of precipitates.[d]	Aniline contained in precipitates.[e]	Gossypol recovered.	
Gm.		*Gm.*	*Per cent.*	*Gm.*	*Per cent.*
[a] 0.7002	Peanut oil	0.8736	26.07	0.6459	92.24
[a] 0.6642	.....do	.8424	25.77	.6253	94.14
[b] 0.6902	Cottonseed oil [c]	.8296	26.50	.6097	88.34
[b] 0.6902	.....do	.8534	27.10	.6221	90.13
[b] 0.6902	.....do	.8594	26.67	.6302	91.37
[b] 0.6902	.....do	.8562	26.67	.6279	90.97

[a] Dried in desiccator over calcium chlorid.
[b] On moisture-free basis, 0.7000 gm. actually taken.
[c] Refined in Oil, Fat, and Wax Laboratory of the Bureau of Chemistry.
[d] Including weight of all subsequent small precipitates.
[e] Calculated on basis of nitrogen analysis.

The error would appear to be on the average about 10 per cent of 0.7 gm. of free gossypol dissolved in 25 cc. of cottonseed oil, equivalent to about 3 mgm. per cubic centimeter of evaporated ether extract. The seeds showing a low gossypol content are not equally poor in oil. There is, therefore, a greater percentage of error in determining the gossypol in the samples of seed running low in gossypol.

A gram sample of gossypol "acetate" gave no weighable ash. Kjeldahl analysis of two preparations of "free" gossypol gave 0.0363 and 0.033 per cent of nitrogen. This small quantity is not significant and undoubtedly represents an impurity in the preparations.

IDENTIFICATION OF GOSSYPOL

Gossypol was identified as the "acetate" in all varieties of cottonseed (Table III) which were analyzed quantitativly, also in Wine Sap (a red-foliage variety), in several samples of gin-run seed, and in Ingenhouzia (Arizona wild cotton). In addition, it was secured from a sample of commercial cotton-root bark. These preparations of gossypol are described as follows, by Dr. Edgar T. Wherry, crystallographer of the Bureau of Chemistry, who found the optical properties to be identical with those of the preparations which were submitted to the writers by Doctor Carruth:

OPTICAL-CRYSTALLOGRAPHIC PROPERTIES OF GOSSYPOL "ACETATE"

All of the samples of gossypol submitted proved to be crystalline, practically insoluble in the usual organic immersion liquids and well adapted for optical-crystallographic study under the microscope. Their properties are as follows:

IN ORDINARY LIGHT.—Consists of bright yellow flakes, often rather acutely rhombic in outline, or sometimes approximately hexagonal. Two or more acute crystals are sometimes grown together to form a twin, with a deep reentrant angle at one end. The crystal system is apparently triclinic.

WITH POLARIZING NICOL.—Pleochroism is very slight, but pseudo-absorption is marked.

REFRACTIVE INDICES.—(20°/D): alpha = 1.530 to 1.540, beta = 1.750 to 1.760, gamma = 1.820 to 1.830, gamma − alpha = 0.290. The grains usually lie in positions oblique to the index directions, so that mean values are shown. By working over large masses of crystal fragments, however, the individual indices can be obtained without great difficulty. The evidence for variation in indices from one preparation to another is definite. This variation, however, is not associated with any other recognizable difference in crystallography, and appears to be due to variation in amount of solvent-of-crystallization or perhaps of material present in solid solution in the crystals.

IN PARALLEL POLARIZED LIGHT, NICOLS CROSSED.—The birefringence is extreme and interference colors are shown only by the thinnest flakes, rarely reaching low orders. Extinction is inclined, the angle varying widely with the orientation of the crystals, but being 20° toward certain frequently occurring edges. Elongation is often negative, but likewise varies with the orientation.

IN CONVERGENT POLARIZED LIGHT, NICOLS CROSSED.—Partial bi-axial interference figures are not difficult to obtain. The axial angle $2E$ is large, probably around 100°. The sign is negative, and dispersion is extremely strong and markedly unsymmetrical.

COMPOSITION OF COTTONSEED

The results of the gossypol determinations, together with those for fat, nitrogen, and moisture content, are given in Table III.

The data in Table III show that the gossypol content of seed may vary by as much as 300 per cent. (Compare Trice, 1918, and Lone Star, 1918, with the Egyptian and Columbia, 1918.) The greatest annual variation in any one variety, approximately 200 per cent, was observed in Trice

seed from Bells, Tenn. (Compare seed of 1917, 1918, and 1919.) Smaller annual variations were noted in certain other seeds, and practically none in others. The authors' series, however, is rather limited, and it is possible that other large annual variations occur frequently in other varieties, particularly in regions which have variable or occasionally unfavorable weather conditions.

TABLE III.—*Gossypol, oil, protein, and moisture content of cottonseed meats*

Variety.	Place grown.	Year grown.	Moisture.	Ether extract.	Nitrogen.	Gossypol found.		
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Lone Star	Greenville, Tex	1917 or 1918	5.93	31.46	6.19	0.4137	0.3833	0.3869
Do.[1]	Yuma Valley, Bard, Calif.[2]	1919	5.64	33.06	5.88	.4636	.4649	....
Do	Greenville, Tex	1919(?)	5.65	33.97	6.30	.5307	.5684	....
Do.[3]	do	1919	....	33.40	6.40	.5288	.5076	....
Do.[4]	Texas	1919	5.45	34.72	5.87	[5].6322	[5].6461	....
Do.[4]	Arkansas	1919	5.84	35.36	5.84	[5].6849	[5].6770	....
Do	Manchester, N. C	1919	5.66	35.45	5.62	.6592	.6899	....
Do.[6]	Courtland, Ala	1919	5.11	38.28	5.45	.7392	.7410	....
Do.[6]	do	1919	6.04	37.13	5.20	.7970	.7982	....
Do.[1]	Bakersfield, Calif	1919	5.68	35.92	5.60	.8781	.8761	....
Do	Elizabeth City, N. C	1919	5.82	38.46	4.91	.9676	....	....
Durango[6]	Courtland, Ala	1919	5.88	37.16	5.27	.7176	.6970	....
Do.[1]	Bakersfield, Calif	1919	5.66	37.01	4.82	.8574	.8709	....
Do	Columbia, S. C	1918	5.70	36.06	5.83	.8736	.9072	....
Do.[6]	Yuma Valley, Bard, Calif.	1919	5.45	37.33	5.13	.8846	.9312	.8831
Do.[1]	do.[2]	1919	5.69	37.40	4.77	.9552	.9319	....
Do.[4]	Georgia, North Carolina, South Carolina.	1919	5.38	38.87	5.19	.9811	.9849	....
Do.[3]	Columbia, S. C	1918(?)	5.93	38.97	4.94	.9843	.9833	....
Do	Columbia, S. C. (?)	1917 or 1918	4.81	40.60	4.88	1.0603	1.0348	....
Trice[3]	Bells, Tenn	1918	6.12	28.87	6.42	.3970	.4250	....
Do	do	1917	5.45	32.51	6.28	.5776	.5797	....
Do	do	1919	6.55	35.84	5.75	.8893	.9061	....
Do[4]	Tennessee	1919	....	....	....	.9426	.9524	....
Do[4]	Eastern States	1919	....	....	....	[5]1.0590	[5]1.1832	....
Acala	Clarksville, Tex	1918(?)	5.44	33.69	6.04	.4374	.4560	....
Do	do	1919	6.21	34.56	5.71	.5554	.5557	.5778
Do.[4]	Oklahoma	1919	4.92	35.41	5.70	.8976	[5].9094	[6].9067
Do.[1]	Bakersfield, Calif	1919	5.25	40.98	4.40	.9639	.9512	....
Meade	Charleston, S. C	1918 or 1919	5.52	37.05	4.97	.5856	.5741	....
Do	Ware County, Ga	1918	5.06	37.87	5.08	.6455	.6446	....
Dixie[4]	Eastern States	1919	5.72	38.27	5.00	[5]1.0185	....	....
Do	Florence, S. C	1919	5.74	39.52	4.66	1.0305	1.0366	....
Columbia	Easley, S. C	1918	5.46	38.89	4.85	1.1105	1.1162	....
Do.[4]	South Carolina	1919	....	....	....	[5]1.0064	[5]1.0278	....
Egyptian[3]	Sacatone, Ariz	1918	5.66	36.68	4.73	1.1832	1.1758	....
Do.[1]	Bakersfield, Calif	1919	5.59	36.08	5.34	1.1745	1.1847	....
King[7]	Richmond, Va	1919(?)	5.24	38.35	5.14	.9219	.9185	....
Cleveland[1]	St. Matthews, S. C	1919(?)	6.49	35.20	5.25	.7127	.6892	....
Sea Island[6]	Blackshear, Ga	1918	....	....	....	[5].9446	....	....

[1] Probably the first year's growth from seeds imported into this region.
[2] Received from the agricultural experiment station.
[3] Used for feeding tests.
[4] The Bureau of Plant Industry distributes seed and receives from planters samples of their trial crops for examination. These seeds were composite samples from a wide range of territory.
[5] Estimated on the basis of 75 per cent gossypol in aniline gossypol.
[6] Received from planter.
[7] Received from seed dealer. Seed probably from immediately adjacent territory.

Table III also shows that the same variety grown in widely separated regions may or may not contain different quantities of gossypol. This indicates that influences other than those of a varietal character play a significant rôle. In direct agreement with this deduction is the observation that different varieties grown in the same region contained approximately the same quantity of gossypol. One exception was Lone Star from Bard, Calif., which was much lower in gossypol than Durango from

the same place. Although not typical of the region from which it came, this sample of Lone Star is in itself not atypical of cottonseed. Egyptian seed from Bakersfield, Calif., contained a slightly larger quantity of gossypol than the three other varieties from this place. This, as well as the high gossypol content of Egyptian seed obtained from Arizona, suggests that there may be slight differences between *Gossypium herbaceum* and *Gossypium hirsutum*, although the single analysis of Sea Island seed does not confirm this.

The results of all the analyses indicate that the occurrence of an intoxication due to gossypol would not be influenced by the variety of seed from which the meal is made, but rather by the place from which it came and the season in which the seed is grown. If a varietal influence upon the gossypol content actually exists, practically it is concealed. The manner of cultivation (agronometric) also probably plays a rôle.

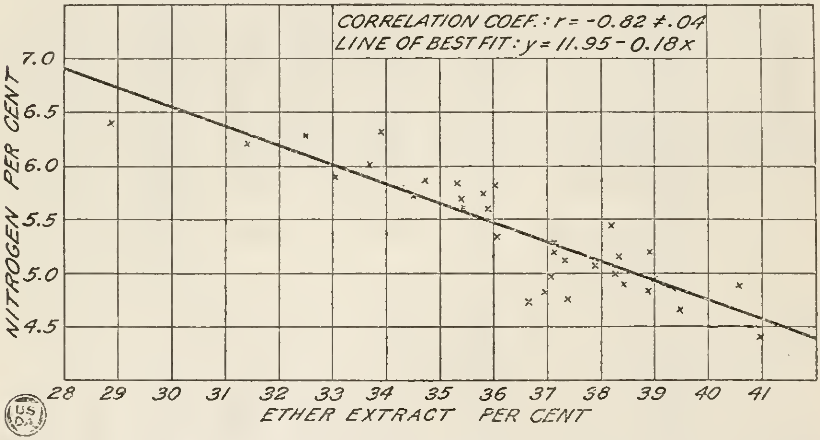

FIG. 1.—Relation between ether extract and nitrogen content of cottonseed. Since r is negative, the nitrogen content decreases as the ether extract content increases. The value of r (0.82), which is large as compared with its probable error (0.04), is significant. The partial correlation coefficient (0.62) indicates a significant relationship between the ether extract and nitrogen content.

Although only presumptive evidence upon this point exists in these experiments, it is a logical supposition to make from the results of Bain and Anders reported by Cook (5).

Figures 1, 2, and 3 give the mathematical expression and interpretation of the writer's data.[7] In these computations each individual gossypol analysis has been used. The correlation coefficients [8] between ether extract (oil) and nitrogen (protein), and between gossypol and ether extract, respectively, show that relationships exist, but that they are not perfect. The reality of these relationships is further borne out by the determination of partial correlation coefficients.

An apparent correlation exists between the nitrogen (protein) and the gossypol; in fact, the results of one analysis may be used to a certain extent to estimate the other. That this relationship may be false is shown by the fact that their partial correlation coefficient is very low.

[7] The calculations were made and the charts were plotted by J. C. Munch.
[8] The correlation coefficient is a measure of the relationship between two variables. It is a relative value, zero, (0) indicating no relationship and unity (1) perfect relationship. The partial correlation coefficient indicates the relationship between two variables when other known variables are eliminated.

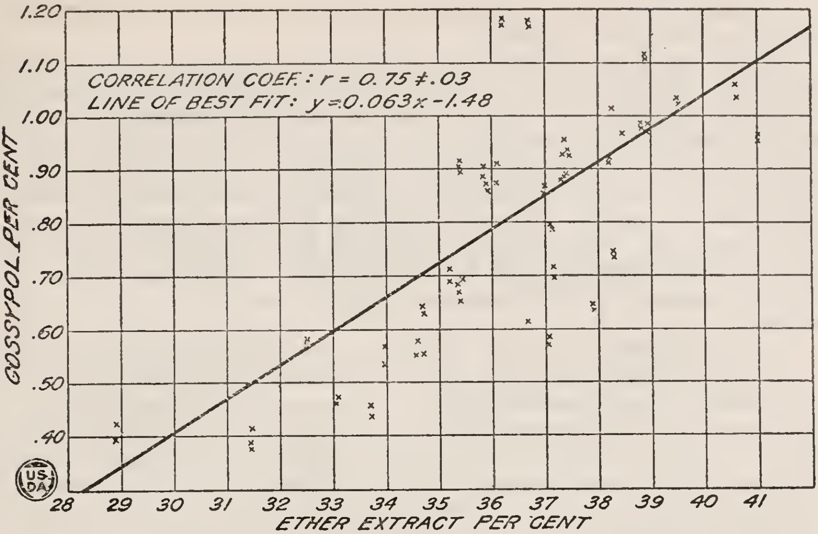

FIG. 2.—Relation between ether extract and gossypol content of cottonseed. Since *r* is positive, the gossypol content increases as the ether extract content increases. The value for *r* (0.75), which is large as compared with its probable error (0.03), is significant. The partial correlation coefficient (0.45) indicates a significant relationship between the ether extract and gossypol content.

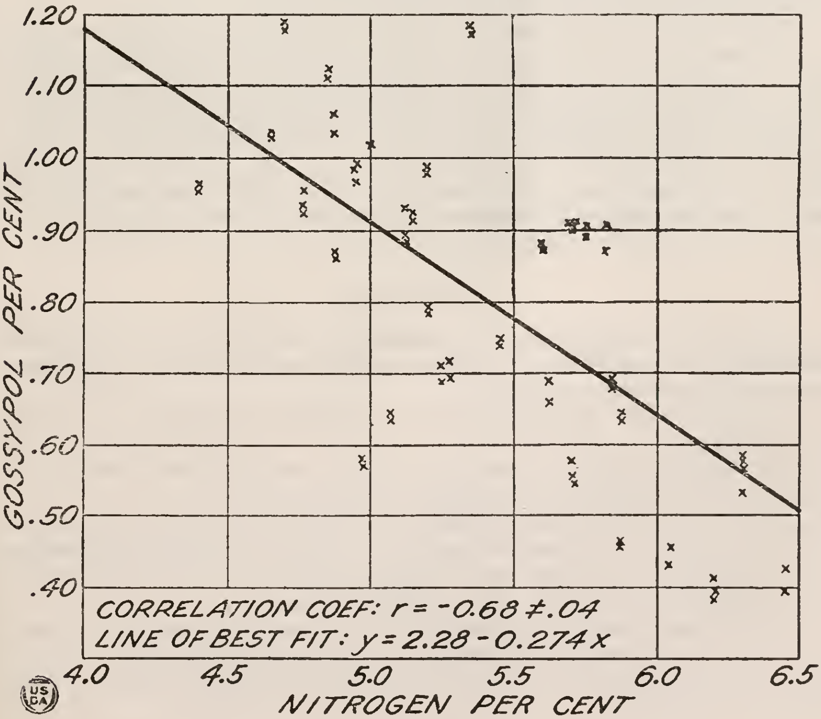

FIG. 3.—Relation between nitrogen and gossypol content of cottonseed. Since *r* is negative, the gossypol content decreases as the nitrogen content increases. The value for *r* (0.68) which is large as compared with its probable error (0.04), is significant. The partial correlation coefficient (0.17) indicates, however, that the correlation may be false.

It is probably true that this apparent relation is due to the close relationship of each of these to the oil.

Before basing generalizations on the gossypol content of cottonseed upon these analyses, it is to be noted that the nitrogen (protein) and the oil content of the seeds fall fairly well within the generalization of Bidwell concerning the interrelation of the quantities of these two constituents in seeds from different parts of the Cotton Belt. This conformity indicates that the small number of samples of seeds analyzed form a representative series. The seeds from the Southwest have a tendency to be low in oil, those from the Southeast to be somewhat higher, and those from the Pacific coast to be still higher. The nitrogen has the reverse relation. The few exceptions which are evident are to be expected.

The analyses show that the seeds from the Southwest tend to be low in gossypol, those from the Southeast somewhat higher, and those from the Pacific coast regions still higher. Even more significant than this is the tendency of the gossypol to follow what may be termed the "rule of the oil." Seeds which are somewhat atypical of the region in which they are grown, as indicated by their oil content, vary correspondingly in their gossypol content. This shows that the gossypol content is closely related to the oil content, and only in a general way to the place of production. The seeds lowest in oil (Lone Star, 1917 or 1918, and Trice, 1918) have the smallest gossypol content, while the seeds highest in oil (Acala from Bakersfield, Calif.) are only 0.2 per cent below the highest in percentage of gossypol (Egyptian seeds).

These results are of interest to plant physiologists. The correlations and variations herein recorded should prove useful in attacking problems dealing with the causes which underlie variation in chemical composition. The possibility of developing a gossypol-free variety of cotton with the retention of the attribute to develop oil, which is at present correlated with the development of gossypol, should be borne in mind. The statement herein made as to the "rule of the oil" should be interpreted to mean the simultaneous correlated appearance of gossypol and oil, and not a cause and effect phenomenon.

SUMMARY

(1) Gossypol was found in the kernels of every sample of cottonseed examined, in Ingenhouzia (Arizona wild cotton), and in a sample of commercial cotton-root bark.

(2) The optical crystallographic properties of gossypol "acetate" are described.

(3) The proportion of gossypol varies in raw cottonseed kernels from about 0.4 to 1.2 per cent, a variation of 300 per cent.

(4) The gossypol content appears to depend upon factors other than varietal factors. If a varietal influence exists, practically it is masked. A variation of 200 per cent was found in samples of one variety from the same plantation, but from crops of different years.

(5) The variation in the gossypol content was fairly regular in that it tended to vary directly with and bore a true relationship to the oil content. This was true for all seeds from any one region, regardless of the regional tendency.

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INHERITANCE OF DWARFING IN MAIZE[1]

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INTRODUCTION

There are two forms of dwarf plants in maize which seem to be inherited as simple Mendelian characters recessive to normal stature. These two dwarf forms differ in most characters but are alike in that the great reduction in stature is brought about in each case through a reduction in the length and not in the number of internodes. One of these dwarfs is known simply as Dwarf (*5*)[2]; the other has been designated Brachytic (*7*).

The variation known as dwarf is one which has been confused until recently with a somewhat similar semidwarf variation now known as anther ear, both being andromonoecious, but that these two variations are wholly unrelated has been demonstrated by the Emersons (*5*). In view of their rather close resemblance, involving the same complex of characters, and the confusion of the two forms in earlier reports, it is not possible, in the absence of genetic comparisons, to state with certainty which of the two forms has been found by the different observers. One of these andromonoecious types of maize was described by Montgomery (*9*), who found it in a stock of Stowell's Evergreen sweet corn. Other independent origins have been reported of variations very similar to the one designated dwarf, which seems to be one of the commonest major variations in maize, appearing in wholly unrelated stocks from widely separated localities.

Two of our pedigreed cultures have given rise to andromonoecious plants of dwarfed stature. These cultures were not related, one being a hybrid of the hairy Mexican type, Esperanza (*2*), with Emerson's liguleless strain (*4*), and the other a variety of maize originally grown by the Pawnee Indians, the seed stock of which was received from Mr. M. R. Gilmore. In this latter case the variations appeared in the fourth generation of consecutive inbreeding. These variations were dissimilar in size, that from the Pawnee variety being somewhat variable in height but obviously larger than that from the Esperanza-liguleless hybrid.

Comparisons were made between these two dwarf forms and the variations described and named by Emerson, anther ear, and dwarf, seed of which was kindly furnished by Professor Emerson. The strain derived from the Esperanza resembles Emerson's dwarf while the dwarf from Pawnee more closely resembles the anther ear, but both variations with respect to stature occupy an intermediate position between anther ear and dwarf, probably due to the height characteristics of their parental strains. The tallest of the dwarf plants, however, are less than half the height of their normal sibs, and while variation exists among them they never approach in height plants of normal stature (Pl. 1). Without

[1] Accepted for publication May 2, 1923.
[2] Reference is made by number (italic) to "Literature cited," p. 320–321.

having tested these variations by interhybridization, it seems certain that at least the smaller of our dwarfs is identical with that known as dwarf by Emerson.

This latter form has been used in the hybrids described in this paper, and to obviate circumlocution is referred to throughout as dwarf. The reader should bear in mind, however, that close somatic resemblance is no certain indication of genetic identity, though the very close similarity of all the teratological characters of these andromonoecious forms raises the question as to whether these variations are not due to some common causes. The andromonoecious dwarfs and semidwarfs seem always to appear as simple Mendelian segregates formed of a complex of characters whose component parts, or at least characters very similar in appearance, are known to occur separately and in different combinations in other nondwarf strains. The most striking characteristic of dwarf is its greatly reduced stature, which often is less than one-fifth that of normal sister plants, although there seems to be no compensating increase in the diameter of the culm. The leaves are reduced in length and increased in width, entirely altering the normal proportions and giving the plants a peculiar tobacco-leaved appearance. Associated with the reduction in stature, there is also a proportional reduction in the size of the tassel, and the number of branches seldom exceeds three. Perfect flowers or staminate spikelets with large anthers are found throughout the ear, which usually terminates in a staminate spike, but, notwithstanding this excess of staminate development, pollen is shed sparingly and the anthers are rarely fully exserted, and even more rarely dehisce. The plants seem exceptionally vigorous and sturdy and the leaves are a very dark green. The reduction in stature is accomplished entirely through a shortening of the internodes—not through a reduction in their number, which remains the same as in normal sister plants. In this respect the plants of dwarf resemble the plants of brachytic, a type of dwarf in which the stature only is reduced (Pl. 2, 3).

The brachytic type also has appeared in unrelated stocks. The chief characteristic of this variation is a shortening of the internodes, which includes also the homologous parts of the tassel, resulting in a reduction of the branching space. This reduction seems unaccompanied by other changes in the tassel, with the possible exception of a slight increase in the number of tassel branches. There are a few other minor changes, such as an increased diameter of the stalk, but nothing of a striking nature comparable to the reduction in stature. The leaves are of the same size and proportion as in normal plants and there is no tendency to produce perfect flowered ears. There is evidence, however, that the brachytic, like the andromonoecious dwarf, is associated with the development of staminate spikes on the ears (*8*).

FIRST GENERATION

In view of their common characteristic of shortened internodes, it might be supposed that crosses between dwarf and brachytic plants would produce nothing but plants of short stature. However, such is not the case, for the first generation of such hybrids consists of normal plants fully as tall as the normal plants from which the immediate brachytic parent is derived, the observed height being 21.0 ± 1.03 dcm. These F_1 plants are also normal with respect to all other teratological characters of their parents. While it is not uncommon to find that

crosses between variations somewhat similar in appearance result in the restoration of the normal form, these two dwarfs are so strikingly alike in the characteristic of reduced internode length that F_1 plants of normal stature were not anticipated.

That the combination of these two dwarf forms should restore completely tall stature furnishes an impressive example of the potential hereditary possibilities resident in abnormal variations and demonstrates the futility of predicting the hereditary behavior of defects which appear similar.

SECOND GENERATION

The distribution to be expected in the second hybrid generation of such a cross, assuming that the two characters are unrelated genetically, is nine normal, three brachytic, three dwarf, and one representing a combination of the dwarf and brachytic forms, the physical characteristics of which can not be predicted from those of the parents. Six rather large progenies were grown, but no group representing the combination of the brachytic and dwarf variation was recognized. The classification of plants is given in Table I.

TABLE I.—*Showing the number and percentages of the three types of plants obtained in the second generation of the dwarf-brachytic hybrid*

Progeny.	Number of—				Percentage of—	
	Normal.	Brachytic.	Dwarf.	Total.	Brachytic.	Dwarf.
Dh 444 L1 R21	123	45	36	204	22.1 ±2.00	17.6±1.8
L2 R21	107	39	17	163	23.9 ±1.90	10.4±1.6
L3 R21	140	60	28	228	26.3 ±2.00	12.3±1.5
L4 R21	145	45	6	196	23.0 ±1.90	3.1± .8
L5 R21	143	40	8	191	20.9 ±2.00	4.2±1.0
L1 R22	560	172	192	924	18.6 ± .27	20.8± .3
Total	1,218	401	287	1,906	21.04± .20	15.1± .17

The first five progenies were grown at Arlington, Va., in 1921, and the sixth at the same place in 1922. Unusual care was exercised in 1922, and both soil and weather conditions were much more favorable for the survival of dwarf plants than they were in 1921. In the discussion to follow only plants raised in 1922 are considered.

Subsequent breeding experiments with self-pollinated brachytic plants from these segregating F_2 progenies have shown that the combination of the two variations resembles dwarf plants very closely, being somewhat smaller perhaps, though not strikingly so, and having the accompanying characteristics such as perfect flowered ears, etc. (Pl. 4). In this respect the double recessive form of brachytic-dwarf differs markedly from that found by Emerson in the cross between dwarf and anther ear, where a strikingly small and rather easily identified sterile double recessive was isolated in the second generation.

Sixteen F_3 progenies were grown from self-pollinated seed of brachytic segregates in the F_2 populations of 1921. Of these, just half proved to be heterozygous for dwarf, these dwarf plants representing the double

recessive combination. While two-thirds or 11 of the 16 would be expected to produce dwarf plants if the brachytic and dwarf characters are independent, the departure of 3 from this expectation may be ascribed to chance. Curiously, the percentage of germination was slightly higher in the segregating progenies than in the others, but not significantly so. The percentage of dwarf plants in the 8 progenies was very close to the expected, though 2 were very low. It is interesting to observe that the percentage of germination on the 3 progenies which were below the expected in the percentage of dwarf plants is 13.8 ± 2.1 lower than the progenies which equaled or exceeded the expected percentage of dwarfs. The classification of plants is shown in Table II.

TABLE II.—*Showing F_3 results obtained from growing self-pollinated seed of the brachytic plants which reappeared in the F_2 of dwarf-brachytic; the dwarf plants obtained represent the combination of the two characters, dwarf and brachytic; the counts are made of seedlings raised in greenhouse flats*

PROGENIES SEGREGATING FOR DWARF

Progeny.	Number of—				Percentage of—	
	Seeds planted.	Non-dwarf.	Dwarf seedlings.	Germinated seeds.	Germination.	Dwarf seedlings.
1	108	49	29	78	72.0	37.2 ± 3.7
2	100	73	27	100	100.0	27.0 ± 3.0
3	106	62	19	81	76.5	23.4 ± 3.2
4	100	65	31	96	96.0	32.3 ± 3.2
5	115	52	18	70	60.9	25.7 ± 3.5
6	100	65	9	74	74.0	12.1 ± 2.5
7	100	52	4	56	56.0	7.1 ± 2.3
8	100	60	29	89	89.0	32.6 ± 3.4
Total	829	478	166	644	77.7	25.8 ± 1.2

PROGENIES NOT SEGREGATING FOR DWARF

Progeny.	Seeds planted.	Non-dwarf.	Dwarf seedlings.	Germinated seeds.	Germination.	Dwarf seedlings.
1	108	96	....	96	89.0	....
2	80	48	....	48	60.0	....
3	117	110	....	110	94.0	....
4	115	93	....	93	81.0	....
5	102	93	....	93	91.0	....
6	115	90	....	90	78.0	....
7	100	10	....	10	10.0	....
8	102	99	....	99	97.0	....
Total	839	639	....	639	76.2	....

The inclusion of the double recessive class in the group of dwarf stature should have been reflected in the ratio of dwarfs to the other groups in the F_2 populations, the expectation then being nine normal, three brachytic, and four dwarf, but it is reasonable to suppose that the relatively high death rate for dwarf plants in field cultures so reduced the percentage of this type that the small increment due to the addition of the double recessive combination did not fully compensate for the loss due to low viability.

When grown under more favorable conditions in greenhouse flats, the percentage of dwarf seedlings, which are recognized easily by their short broad leaves, is found to approximate closely the expected 25. Thus three progenies involving 823 plants gave, respectively, 31.4 ± 2.4, 25.1 ± 1.74, and 21.3 ± 1.43, with a percentage of 24.7 ± 1.02 for the combined totals.

Measurements were made of five characters of 924 plants of the second generation progeny grown at Arlington in 1922. These plants also were classified arbitrarily into the three groups of normal, dwarf, and brachytic stature. In addition, the plants were classed for pericarp color, anthers in the ear, and staminate spikes on the ear. These latter might better have been measured, since they varied greatly in length and in the ratio of the staminate to pistillate portions. The hybrids also involved liguleless leaves and were classed with respect to this character. The biometrical constants of these characters are given in Table III and the percentages of individuals showing them in the three groups and in the total population are given in Table IV.

TABLE III.—*Biometrical constants for the second generation of the dwarf-brachytic hybrid grown at Arlington, Va., 1922*

	Stature.									Total population.		
	Normal.			Dwarf.			Brachytic.					
	Mean.	σ	PE.	Mean.	σ	PE.	Mean.	σ	PE.	Mean.	σ	PE.
Height	23.3	4.00	0.12	4.87	0.99	0.05	8.55	1.97	0.10	16.70	8.86	0.20
Length fifth leaf	77.0	10.30	.30	40.06	8.30	.42	67.66	1.12	.08	72.60	17.90	.42
Width fifth leaf	9.9	1.96	.06	11.20	2.19	.11	8.89	2.04	.13	10.10	2.10	.05
Number tassel branches	18.3	10.50	.30	.69	1.30	.06	20.19	11.40	.65	14.80	12.10	.28
Total number leaves	22.5	1.60	.05	24.70	2.00	.27	25.04	2.02	1.57	22.96	1.99	.06
Leaf index	12.2	3.10	.09	25.17	3.90	.20	12.66	4.51	.29	15.10	6.38	.15

TABLE IV.—*Percentage of plants in the three stature groups having the characters listed in column 1*

Characters.	Stature.						Total population.	
	Normal.		Dwarf.		Brachytic.			
	Per cent.	*PE.*	*Per cent.*	*PE.*	*Per cent.*	*PE.*	*Per cent.*	*PE.*
Brachytic	....	....	....	....	....	....	18.7	0.87
Dwarf	....	....	....	....	....	....	20.8	.91
Liguleless	27.0	1.26	20.3	1.94	27.9	2.3	25.8	.97
Perfect flowered ears	.6	.22	100.00	....	4.55	1.21	22.95	.98
Staminate spikes	26.2	1.28	99.9	....	18.2	1.45	40.9	1.14
White pericarp	20.9	1.65	22.7	4.2	40.3	4.36	24.1	1.49
Tassels without branches	2.2	.42	67.8	2.3	2.9	.58	16.2	.84

INHERITANCE OF SIZE CHARACTERS

As was to be expected, the height of the plants as measured in decimeters showed a trimodal distribution, the dwarf and brachytic plants forming a group at the low end of the scale. The frequency distribution for this character is shown in figure 1 and their relative heights are shown in Plate 5.

In the cross between dwarf and anther ear, reported by the Emersons, where normal stature also was restored in the F_1, the distribution with respect to height shows the anther ear and normal segregates to be more

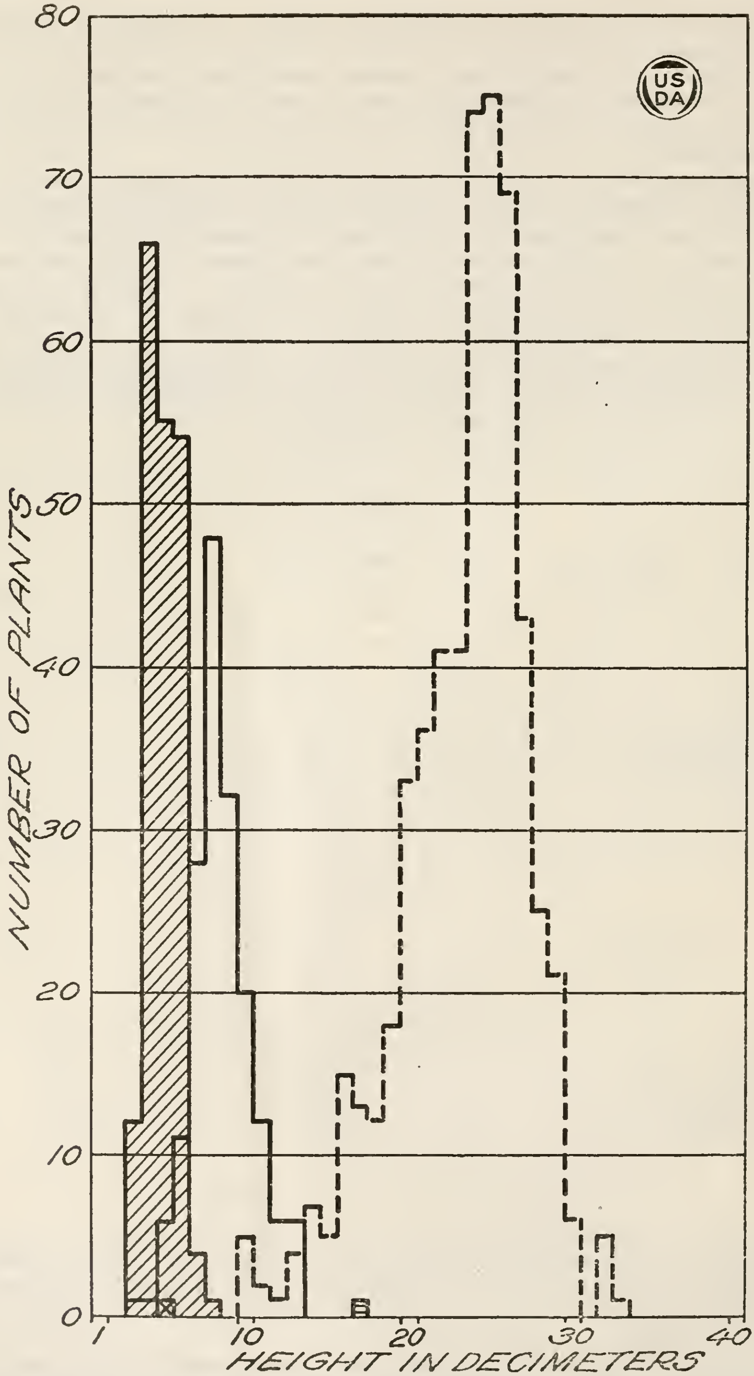

FIG. 1.—Frequency distributions for plant height. Shaded portion, dwarf plants; solid lines, brachytic plants; broken lines, normal plants. The square marked X represents a plant classed as normal; the square with horizontal lines represents a plant classed as brachytic.

nearly of the same height, while the dwarf segregates are much smaller with no overlapping (5). This is different from the brachytic-dwarf hybrid, where the brachytic and dwarf segregates form one group and the normals the other. An examination of the height data shows that brachytic occupies with respect to stature a position intermediate between anther ear and dwarf, approaching the dwarf stature rather more closely

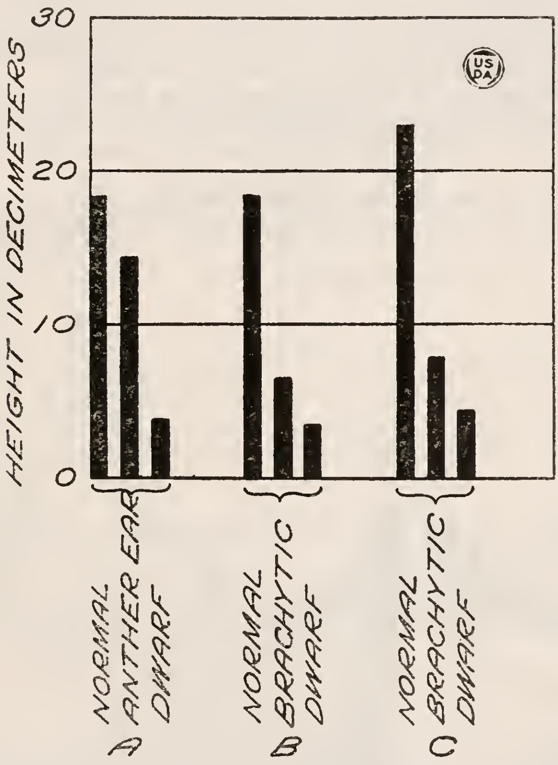

FIG. 2.—Comparison of the plant heights of normal, anther ear, and dwarf as grown by the Emersons, with normal, brachytic, and dwarf from the dwarf-brachytic hybrid. A, Average height of Emersons' normal, anther ear, and dwarf plants. B, Average height of normal, brachytic, and dwarf plant from the dwarf-brachytic hybrid reduced in proportion to the difference between the normal segregates of the two hybrids. C, Average height of normal, brachytic, and dwarf plants from the dwarf-brachytic hybrid.

than the anther ear. This relationship is shown diagrammatically in figure 2.

The entire population of the brachytic-dwarf hybrid was taller than that of Emerson's anther ear dwarf, but in reducing each stature group of the brachtyic-dwarf hybrid in proportion to the difference between the normal segregates of the two hybrids, the groups of dwarf stature are found to be very similar, the mean height being 3 and 3.87, respectively, while the mean height of the brachytic group becomes 6.8 as compared with 14.8 for the anther ear stature.[3]

[3] Three F_2 populations are shown in the Emersons' paper. Two of these populations are very similar in the mean height of the three groups, while the other (the first) is somewhat smaller. In making the height comparisous, the group having the tallest plants of normal stature was chosen; this population also had the largest number of individuals but the relationships of the three height groups in anther-ear dwarf to those of the brachytic-dwarf would remain very much the same irrespective of which of the three anther-ear dwarf groups was selected.

The length of the fifth leaf from the top also showed a good bimodal distribution with the brachytic plants, forming a somewhat intermediate grouping, as shown in figure 3.

The width of the fifth leaf was strictly unimodal, with the dwarf plants grouped at the upper end of the scale. There was little difference between the normal and brachytic plants with respect to this character, as is shown in figure 4.

From the length and width of the fifth leaf it was possible to formulate an expression for leaf shape. This figure has been designated leaf index and was obtained by dividing the width by the length.

The distribution of plants with respect to leaf shape was bimodal, with the dwarf class well grouped at the upper end of the scale and the brachytic and normal plants occupying the lower end as shown in figure 5.

With respect to the number of tassel branches, 68 per cent of the dwarf plants had no branches and the highest number of tassel branches found

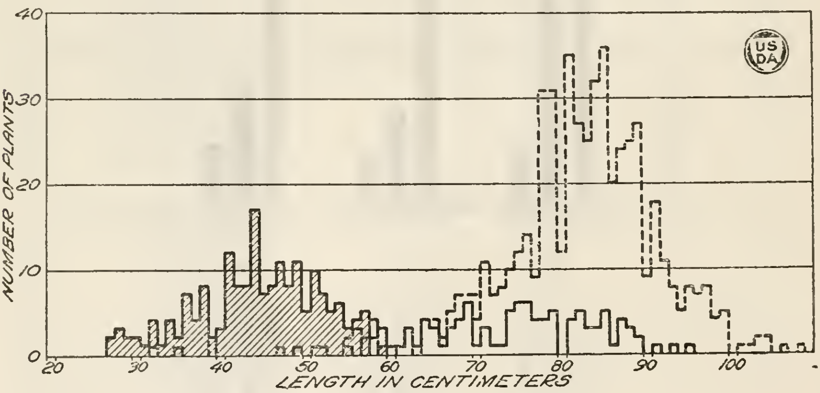

FIG. 3.—Frequency distribution for length of fifth leaf. Shaded portion, dwarf plants; solid lines, brachytic plants; broken lines, normal plants.

in this group was 6, while the brachytic plants ranged from 0 to 57 branches and the normals from 0 to 44, the latter forming a fairly regular distribution, as is shown in figure 6.

The brachytic and dwarf plants differed little in total number of leaves, though both had an appreciably higher number than the normal plants. The distribution, however, was unimodal, as is shown in figure 7.

The relative differences between the segregates with respect to all measured characters is shown in figure 8.

From the character of these distributions it would seem that in the case of the dwarf variation a relatively few hereditary elements will account for the differences between this and the normal form. The behavior of the leaf lengths and shapes is very different from that encountered where short, broad leaves of nondwarf stock are crossed with relatively long, slender leaves of some other strain. In such cases the frequency distributions are unimodal, the indication being that several hereditary factors are concerned in the differences between the parents.

CORRELATIONS OF MEASURED CHARACTERS

The character of the distributions in most cases precludes the use of the correlation coefficient, since the measured character so often is bimodal. Recourse may be had to fourfold groupings, dividing the population arbitrarily into two groups of the measured character, but when such

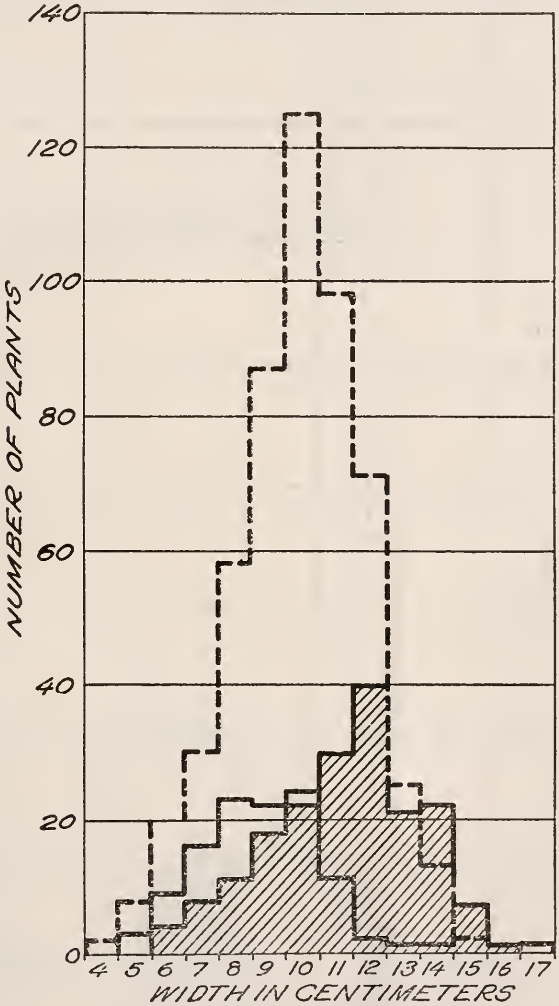

Fig. 4.—Frequency distribution for width of leaf. Shaded portion, dwarf plants; solid lines, brachytic plants; broken lines, normal plants.

divisions are made with due regard to the character of the distributions one class is often zero or very small. Under such conditions a correlation coefficient is practically without meaning, and such coefficients have not been calculated. In those cases where the data justified the use of the biserial correlation, the coefficients have been calculated and are given in Table V. For the most part, however, the frequency polygons will give a clear conception of the nature of the inheritance.

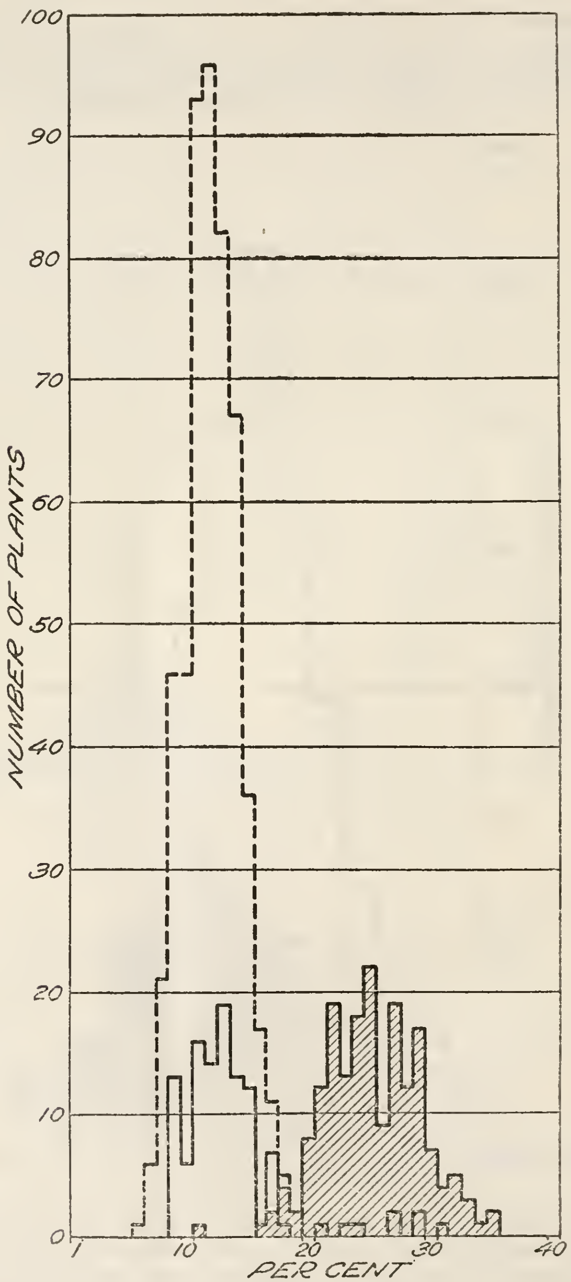

FIG. 5.—Frequency distribution ;or leaf index. Shaded portion, dwarf plants; solid lines , brachyt i plants; broken lines, normal plants.

TABLE V.—*Coefficients of biserial correlations in the second generation of the dwarf-brachytic hybrid*

Measured characters.	Stature groups.		
	Normal versus brachytic stature.	Normal versus dwarf stature.[b]	Dwarf versus brachytic stature.
Length leaf	[a]−0.40±.033		
Width leaf	[a]− .20±.037	0.37±.029	0.431±.037
Number tassel branches	.12±.036		
Total number leaves	.75±.045	.09±.063	[c]−.099±.054
Leaf index	.22±.037		
Height			[c]−.882±.035

[a] Minus sign indicates negative correlation with brachytic stature.
[b] Correlations are positive with dwarf stature.
[c] Minus sign indicates negative correlation with dwarf stature.

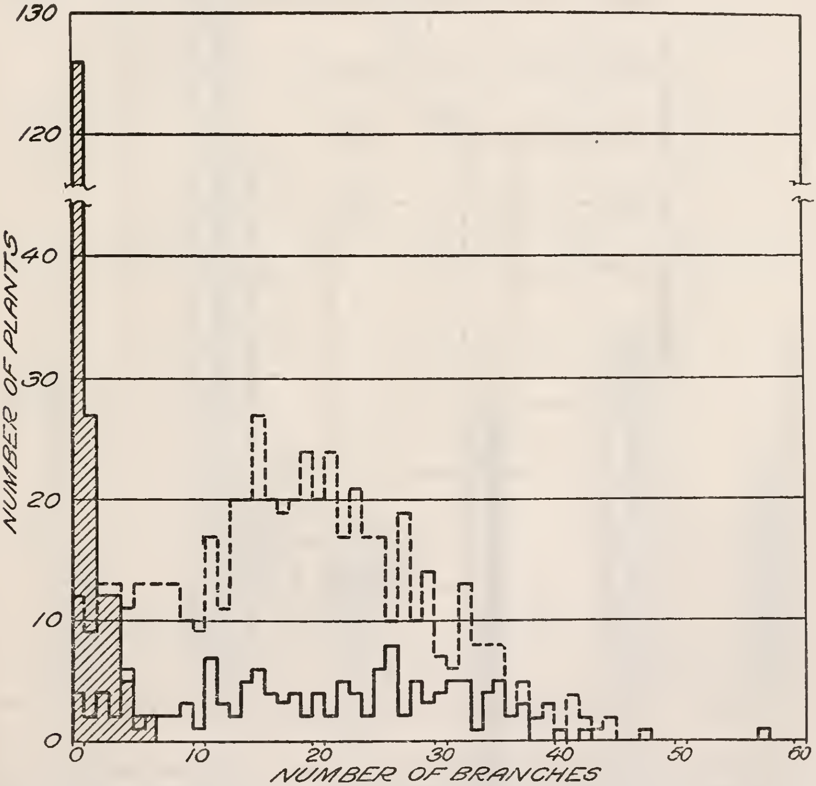

FIG. 6.—Frequency distribution for number of tassel beanches. Shaded portion, dwarf plants; solid lines, brachytic plants; broken lines, normal plants.

While the heterogeneous nature of the F_2 plants precludes the possibility of analyzing the correlations of the measured characters in the population as a whole, some insight may be gained on the interrelations of these characters if the three groups of plants, normal, brachytic, and dwarf, are analyzed separately. These correlations are given in Table VI.

In each of the three groups of stature, tall plants are found associated with long leaves, a relation to be expected from the standpoint both of physiology and genetics. It is of interest, however, that the relationship is no closer, especially in the group of normal stature.

With leaf width, height is found to be negatively correlated in the brachytic group, while the coefficients of correlation in the other two groups are essentially the same as for leaf length. The negative correla-

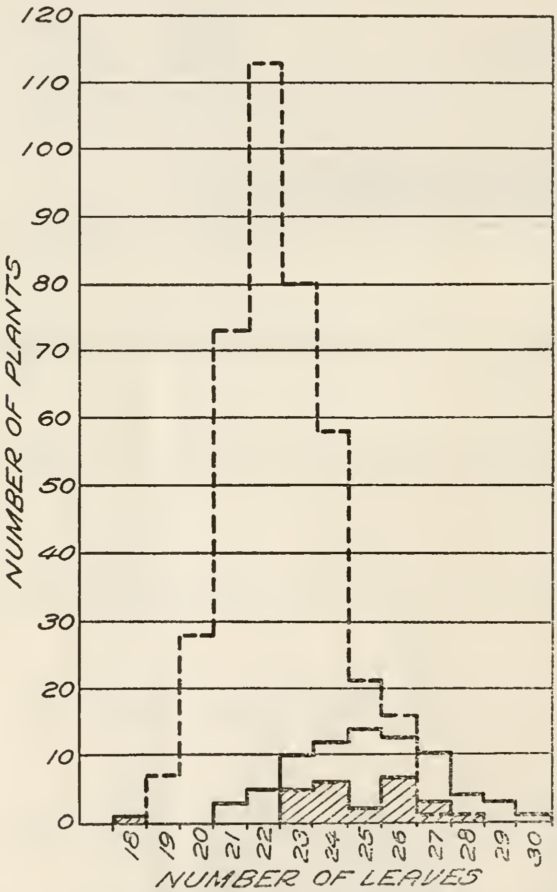

FIG. 7.—Frequency distribution for total number of leaves. Shaded portion, dwarf plants; solid lines, brachytic plants; broken lines, normal plants.

tion in the brachytic group indicates that some sort of segregation of the dwarf stature and wide leaves is taking place in this group, since a positive correlation would be expected if the association were due to physiological causes. The correlation with leaf index indicates that the leaves were broad, not only absolutely but relatively, this fact being in the nature of a substantiation of the hypothesis that some of the plants classed as brachytic were potentially dwarfs with respect to these characters.

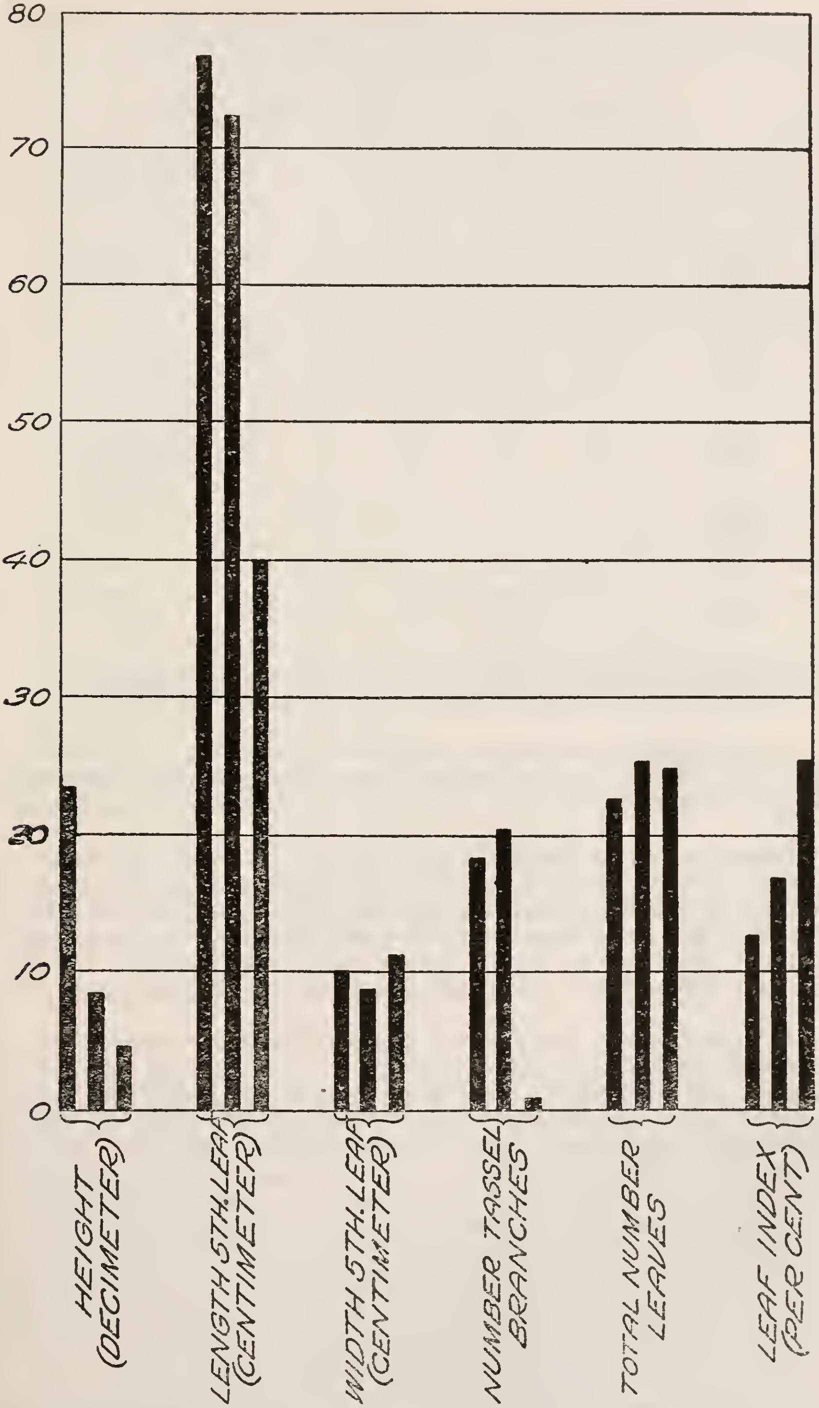

FIG. 8.—Diagrammatic representation of the differences between the means of measured characters in the second generation of the dwarf-brachytic hybrid. Normals at left, brachytic in center, dwarf at right.

TABLE VI.—*Correlation coefficients of the measured characters in the three groups of F_2 plants of the dwarf-brachytic hybrid, normal, brachytic, and dwarf*

	Height.	Length fifth leaf.	Width fifth leaf.	Number tassel branches.	Total number leaves.	Leaf index.	Stature.
Height.		0.215 .434 .441	0.217 −.281 .399	−0.045 −.071 .164	0.161	0.109 −.476 −.045	Normal.[1] Brachytic.[2] Dwarf.[3]
Length fifth leaf.	.215 .434 .441		.255 −.161 .620	.241 .462 .205	−.100	−.393 −.672 −.261	Normal. Brachytic. Dwarf.
Width fifth leaf.	.217 −.281 .399	.255 −.161 .620		.422 .189 .148	.118	.618 .782 .496	Normal. Brachytic. Dwarf.
Number tassel branches.	−.045 −.071 .164	.241 .462 .205	.422 .189 .148		.032	.197 −.192 −.148	Normal. Brachytic. Dwarf.
Total number leaves.	.161	−.100	.118	.032		.142	Normal.
Leaf index.	.109 −.476 −.045	−.393 −.672 −.261	.618 .782 .496	.197 −.192 −.148	.142		Normal. Brachytic. Dwarf.

[1] In the group of normal stature coefficients above 0.103 are greater than three times their error.
[2] In the group of brachytic stature coefficients above 0.190 are greater than three times their error.
[3] In the group of dwarf stature coefficients above 0.147 are greater than three times their error.

With no genetic interference, wide leaves are found to be correlated with tall plants. In the present hybrid the parental combinations were the reverse of this association and the short plants had the wide leaves.

When the biserial correlations of type of plant with leaf width are examined it is found that as between normal and dwarf stature, short stature is correlated with wide leaves, 0.37 ± 0.029, and as between brachytic and dwarf short stature is associated with wide leaves, 0.43 ± 0.037. When the two stature groups, normal and brachytic, are compared it is found that tall stature is correlated with wide leaves, 0.2 ± 0.037.

As stated above, the product moment correlation between stature and leaf width in the brachytic group is -0.281 ± 0.045 between tall stature and wide leaves. Obviously, such an association can be only genetic, since a normal physiological behavior would lead to a positive correlation between tall plants and wide leaves. There can be little doubt that a genetic correlation of this sort must be reduced in degree by an amount proportional to the positive correlation expected for physiological reasons. In this case the normal interaction of physiological factors in the brachytic group would be expected to be at least as high as that found in the normal group, which is 0.217 ± 0.033, so that the effect of the genetic factors must be the difference between the correlation observed in the normal group and that found in the brachytic group, or 0.498, which is a rather high degree of relationship.

Such a result indicates that in the brachytic group there are segregating height factors resembling dwarf in that they are associated also

with factors for broad leaves. This would be brought about if dwarf plants were included among the brachytic plants in the classification into groups. The frequency polygon for stature in the brachytic group shows clearly that there is no bimodality, and an examination of the short, broad-leaved plants in this group fails to show any of the other dwarf characters, such as few tassel branches or perfect flowered ears, eliminating the possibility that the plants were classified improperly.

A further substantiation of the dwarflike characteristics of certain brachytic plants is found in the negative correlation between length and width of leaf, a correlation which is positive in both the normal and dwarf groups. Almost as unexpected is the rather high positive correlation of length with width of leaf in the dwarf group, the group which as a whole has short wide leaves. The restoration of the normal physiological correlation indicates that there is no segregation of varying degrees of dwarfness as such in this group, but rather that such variation as exists is due to the effects of environment or possibly to unrelated modifying factors.

The correlation of width of leaf with number of tassel branches in both the dwarf and brachytic groups are much smaller than the correlation in the normal group, though all three are positive. The coefficients in the brachytic and dwarf groups do not depart significantly from zero, while the coefficient in the normal group clearly is significant. The fact that the normal physiological relationship of wide leaves and many tassel branches has been largely reduced in the brachytic and dwarf groups indicates that a genetic cause is involved, an indication which derives support, of course, from the fact that the dwarf parent represents a condition where not only relatively wide but actually wide leaves are associated with few tassel branches.

The number of tassel branches shows no other unusual relationhips. The correlations with total number of leaves were not calculated for other than the normal group, since on both brachytic and dwarf plants a large number of leaf tags were lost.

Thus the population of these groups was reduced, and since the loss of tags was in inverse proportion to the height of the plants a selective action was involved which might result in spurious relationships so that correlation coefficients would be of little value if calculated.

INHERITANCE OF TERATOLOGICAL VARIATIONS

STAMINATE EAR SPIKES

The percentage of plants with staminate ear spikes in the whole F_2 population, including normal, brachytic, and dwarf plants, is 40.9 ± 1.14. The three groups of stature—normal, brachytic, and dwarf, had, respectively, 26.2 ± 1.28, 18.2 ± 2.27, and 99.4 ± 0.4 per cent of the plants with staminate spikes.

The difference in percentage of this character between the normal and brachytic groups is 8.0 ± 2.61, or 3.06 times the error. Yule's coefficient of association between normal stature and the development of staminate spikes in the normal-brachytic population is only 0.229 ± 0.074, while the departure from a 50 per cent crossover ratio as measured by χ^2 is 3.71. It may be concluded, therefore, that the brachytic and normal groups are alike with respect to the development of staminate ear spikes, both approximating 25 per cent. The hypothesis has been advanced

previously that the development of staminate ear spikes is dependent upon the interaction of two factors, the character appearing when either or both factors are homozygous recessive (*8*). In addition, one of these factors is associated or linked with brachytic, while the other is independent of stature.

The percentages of this character in the three stature groups of the present hybrid necessitates the assumption of a third element for the production of staminate spikes. This third element is linked closely with dwarf stature.

Such characters are generally considered as multiple factor characters comparable with those which come into expression only when all the factors are homozygous recessive. There is little to justify such a classification except perhaps an inability to distinguish somatic differences, an inability which admittedly is personal.

With cases such as the aleurone color of the seeds, where all white seeds have very much the same shade, there is little hope of distinguishing a difference between the several factors and little is to be gained by considering each of the several forms of genetic whites as separate monohybrid characters. With other characters where distinctions are not made in the beginning they are often recognized later, and the variations are then classified as independent monohybrid characters.

It would seem best to consider those characters which come into expression when single or when each of several factors is homozygous recessive, as distinct monohybrid characters, even though they can not be distinguished readily; whereas those characters which appear only when more than one factor is homozygous recessive are true multiple factor characters. With the former each variation in the germ plasm results in a visible somatic change, while in the latter a somatic change results only from the cumulation of several variations in the germ plasm.

It is obvious that a cross involving two independent monohybrid characters which are indistinguishable in appearance would result in the familiar 9:7 dihybrid ratio in the second generation, and the present hybrid may illustrate such a case.

It is clear that the dwarf-brachytic hybrid is homozygous dominant for the factor for staminate ear spike which is linked with brachytic and heterozygous for a factor independent of stature. If this were all, then monohybrid ratios would be expected for the entire population as well as for each stature group, but if a second staminate ear spike character were involved, the gene for the latter being identical or closely linked with dwarf stature, the observed percentages would be approximated closely.

On this hypothesis 25 per cent of the plants in both the normal and brachytic groups would be expected to have staminate ear spikes while all the plants of dwarf stature would have this character, and the percentage for the total population would be 43.75, provided the three stature groups were present in the expected ratio of 9:3:4.

If this hypothesis be true, there are then four staminate ear spike characters, all similar in appearance but distinct genetically. One of these is linked closely with dwarf stature, one is associated with the anther ear semidwarf of Emerson, one is linked with brachytic culms, and the other seems independent of stature.

Variations such as these, strikingly similar in appearance, which prove to be distinct genetically when crossed, are being found with increasing frequency in maize. When such variations affect a similar and peculiar

combination of characters, as is the case with anther ear and dwarf or dwarf and brachytic, the question of a common cause can hardly be avoided. From the mode of inheritance of these characters there can be no doubt that independent genetic changes have taken place on separate chromosomes. That such strikingly similar somatic resemblances can arise as the result of the alteration of unrelated and wholly separate hereditary elements appears incredible. It seems more probable that in maize some or all of the 10 chromosomes are practically identical, each with hereditary elements for all the characters of the complete plant as suggested by Emerson (*3*). Such a hypothesis permits the prediction that all characters in maize eventually will be found to involve at least 10 independent factors and that these factors in a general way will have similar linkage relations.

Thus the shortened internodes of dwarf and brachytic are genetically distinct, and both are associated with the development of staminate ear spikes. These spikes, like the shortened internodes, though bearing a close resemblance to each other, are wholly distinct from a genetic standpoint. Even more striking, of course, is the character complex of anther ear and dwarf. It seems not unreasonable that a change of whatever form in a similar group of genes in separate chromosomes would result in a somewhat similar somatic behavior. Such a hypothesis is in accord with the fact that most linked groups involve diverse organs and that the factors or hereditary elements for multiple factor characters are not found in one chromosome but are distributed through many.

If other organisms possess identical chromosomes, then those organisms with few chromosomes should have relatively simple characters, speaking in a genetic sense, while those with many chromosomes should have complex characters composed of many factors. If mutations occur at the same rate in organisms with few chromosomes, as in those with many, then those organisms with few chromosomes should have many multiple allelomorphs while those with many chromosomes should have multiple factor characters.

PERFECT FLOWERED EARS

Perfect flowered ears always have been found associated with the dwarf stature in its numerous appearances. The character is of more than usual interest since it represents a reversion toward a more primitive form of maize, comparable in some respects to the ramose and tunicate forms. Perfect flowered ears are not limited to dwarf plants, and many maize breeders have encountered them on plants otherwise quite normal. In the writer's experience the character is ephemeral, repeated inbreeding failing to stabilize its appearance in stocks of normal stature; and since, strictly speaking, it is the development of vestigial floral organs, it may well be that environment, especially photoperiodism, which is recognized as having a profound influence on the development of sex organs, is an important factor in its expression.

In the present hybrid, 22.95 ± 0.98 per cent of the plants were found to have perfect flowered ears. This seems fairly close to the expected 25 per cent and it may be accepted as a simple Mendelian character. When the three classes of plants—normal, brachytic, and dwarf—are examined with respect to this character, it is found that of the normal plants only 0.6 ± 0.22 per cent have perfect flowered ears, while of the

brachytics 4.5 ± 1.2 and of the dwarfs all have perfect flowered ears. The distributions are:

	Pistillate ears.	Perfect flowered ears.	Total.	Per cent.
Normal stature	532	3	535	0.56 ± 0.22
Brachytic stature	126	6	132	4.54 ± 1.2
Dwarf stature	0	187	187	$100.00 \pm$
Total	658	196	854	$23.0 \pm .98$

Combining the brachytic and normal stature groups, which differ only slightly in excess of three times the error in the percentage of plants with perfect flowered ears, the resulting fourfold distribution is:

	Pistillate ears.	Perfect flowered ears.	Total.
Nondwarf	658	9	667
Dwarf	0	187	187
Total	658	196	854

Looked at as closely linked but distinct characters, Haldane's $P = 0.9888$ with the percentage of crossover 1.12 (*6*). The expected distribution on the assumption of a 1.12 per cent crossover is:

Expected	636.3	4.2	4.2	209.3
Observed	658.0	0	9	187.0
	22.0	4.2	4.8	22.0

$\chi^2 = 12.75$; this is a rather poor fit, but in this case the departure from the Mendelian percentages are being measured also, and there is direct evidence that the population has been reduced through the low viability of the dwarf plants. With this knowledge, it seems quite fair to calculate the expected on the basis of the actual percentage of dwarf plants found, since the end result will afford an opportunity to determine whether the absence of crossover plants in the dwarf group is of any significance.

The distributions, taking into account the low percentage of dwarf plants, become—

Calculated	661.1	6	3.7	183.3	
Observed	658.0	9	0	187.0	
	3.1	3.	3.7	3.7	($\chi^2 = 5.287$.)

The fit is rather better, and it seems safe to conclude that the genes for dwarf stature and perfect flowered ears are located in the same chromosome with approximately 1 per cent of crossing over.

The failure to find a crossover class involving the short stature of dwarf suggests that such combinations fail to survive, though such an hypothesis seems wholly without a logical basis, since all crossovers in this group would be in the direction of normal plants and, therefore, theoretically have a higher survival value than the noncrossover, unless the assumption is made that some or one of the characters associated with dwarf stature is closely linked with dominant factors for growth, and hence the crossovers with stature would have still a lower survival value than the dwarf complex. In some aspects this hypothesis seems worthy of consideration.

Lethal or semilethal characters that recur constantly must be due either to frequent mutation or, as seems more probable, must be associated with some hereditary element that raises the survival value of the heterozygous stock. While mutations are known to occur in certain stocks with frequency, if such mutations have a negative survival value only, it is difficult to see how they and their mutating stocks persist, since their lethal nature insures their rapid elimination. That stocks of maize heterozygous for lethal characters are common is well known. Perhaps the most widely recognized of these is the albino, or white seedling, which, of course, never produces seed. There are others not so well understood but equally lethal, while the number of semilethals seems legion. Perhaps the most common of these, from the standpoint of repeated occurrence, is the andromonoecious dwarf. If, as has been suggested, maize possesses dominant factors favorable for growth, the problem of the recurrence of lethal factors is simplified by predicating that the deleterious variations which reappear frequently are those closely linked with one or more of these factors for growth, though the mutations need not have arisen in the chromosomes with the favorable growth factors since occasional crossovers would permit their survival. On this hypothesis it seems clear that lethal or semilethal variations must be those closely linked with the factors favorable for growth and, therefore, though destined for death in the homozygous condition, have a survival value as heterozygotes higher than that of normal plants. If this be true, then in a stock heterozygous for lethal or semilethal factors, the homozygous normal plants should be inferior in reproductive value to the heterozygous ones.

Breeders long have recognized that certain variations have a sturdy, vigorous appearance which belies their inherent defects. Combinations of such variations usually make very favorable first generation hybrids, and it should be possible to reconstruct a high yielding strain by combining the deleterious recessive variations. If this be true generally, the practice of inbreeding and discarding those progenies which show deleterious Mendelian variations is resulting in the elimination from the stock of the most desirable hereditary elements.

The survival value of dwarf plants under field conditions is so low that a higher death rate for the crossover classes in this group could pass unnoticed, and of course there is no possibility of recognizing them in the seedling stage.

In an unrelated hybrid between Esperanza and liguleless, the combination of pistillate ear and dwarf stature was obtained. The fourfold distribution in this hybrid is:

Normal stature, pistillate ears.	Normal stature, perfect flowered ears.	Dwarf stature, pistillate ears.	Dwarf stature, perfect flowered ears.
210	10	4	48

The percentage of perfect flowered ears is 21.3 ± 1.67 and the percentage of dwarf plants is 19.1 ± 1.6, while $Q = 0.992 \pm 0.004$, indicating about 5 per cent of crossing over.

LIGULELESS LEAVES

The percentage of plants with liguleless leaves in the entire population was 25.8 ± 0.97, while the percentage in the normal group was 27.0 ± 1.27, in the brachytic 27.9 ± 2.3, and in the dwarf 20.3 ± 1.94. Since the percentage for the entire population closely approximates the expected, the low percentage of liguleless plants in the dwarf group can not be charged to a low survival value of the dwarf-liguleless combination. There is little evidence, however, of a linkage, since $Q = 0.2 \pm 0.06$ between dwarf stature and normal leaves, the parental class being dwarf stature and liguleless leaves. The percentage of liguleless plants in the combined normal and brachytic group is 27.2 ± 1.1, from which 20.3 ± 1.94 differ by 6.9 ± 2.23, or but slightly in excess of three times the error.

While there seems to be no association between stature and liguleless leaves, there is a pronounced correlation between liguleless leaves and few tassel branches. This correlation is found in all three of the stature groups, being greatest in the brachytic and least in the dwarf. Such a correlation is in the nature of a coherence and may indicate a linkage between these characters, one of which is certainly very closely associated with dwarf stature. The frequency distribution for number of tassel branches with respect to normal and liguleless plants of tall stature is shown in Figure 9.

TABLE VII.—*Biserial correlations with liguleless leaves in the second generation of dwarf-brachytic*

Characters correlated with liguleless.	Population of—		
	Normal stature.	Brachytic stature.	Dwarf stature.
Height of plant	[a] -0.097 ± 0.023	0.031 ± 0.040	0.193 ± 0.040
Length fifth leaf	$-.015 \pm .023$	$-.404 \pm .048$	$-.346 \pm .041$
Width fifth leaf	$-.339 \pm .023$	$-.306 \pm .047$	$-.302 \pm .040$
Number of tassel branches	$-.500 \pm .023$	$-.629 \pm .045$	$-.274 \pm .040$
Total number of leaves	$-.009 \pm .027$	$.116 \pm .061$	$.269 \pm .112$
Leaf index	$-.191 \pm .023$	$-.093 \pm .048$	$-.227 \pm .041$

[a] Minus sign indicates a negative correlation with liguleless leaves.

Negative correlations are indicated between leaf width and liguleless leaves in all three groups, and in the brachytic and dwarf groups a correlation with length of leaf is found. The correlation with leaf width is in

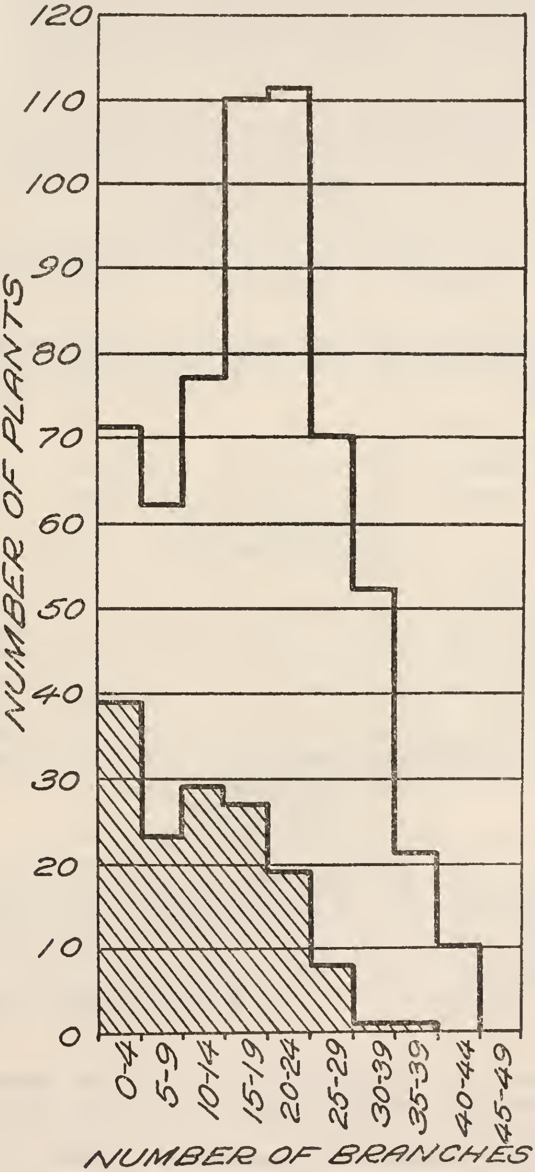

Fig. 9.—Frequency distributions with respect to number of tassel branches on liguleless and nonliguleless plants. All plants of normal stature. Shaded portion, liguleless plants; mean normal, 20.6±0.33; mean liguleless, 11.8±0.45; difference, 8.8±0.56; D/E=15.7; biserial r=−0.50.

the nature of a disherence, since the parental combination was liguleless and wide-leafed. Disherences of this nature where deleterious variations are combined with characters of diminished size indicate some

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physiological cause. Thus, crosses between short plants with broad leaves and tall plants with narrow leaves usually show in the F_2 a correlation between tall plants and wide leaves—a correlation easily understood. Some such explanation may apply in other cases of disherence where the physiological relationships are not indicated so clearly, but are working at cross purposes with genetic relationships. The correlation coefficients are given in Table VII and the biometrical constants in Table VIII.

TABLE VIII.—*Biometrical constants for plants with liguleless and normal leaves in the three stature groups, normal, brachytic, and dwarf*

NORMAL STATURE

	Normal leaves.			Liguleless leaves.			Difference.	D/E.
	Mean.	σ	PE.	Mean.	σ	PE.		
Height	23. 11	3. 81	0. 13	23. 77	4. 54	0. 25	+0. 66±0. 29	2. 3
Length fifth leaf	77. 10	9. 85	. 34	76. 80	10. 83	. 63	—. 30± . 72	. 4
Width fifth leaf	10. 19	1. 95	. 13	9. 07	1. 76	. 10	—1. 12± . 16	7. 0
Number tassel branches	20. 62	10. 29	. 33	11. 80	8. 14	. 45	—8. 82± . 56	15. 7
Total number leaves	22. 52	1. 57	. 10	22. 28	1. 54	. 11	—. 24± . 15	1. 6
Leaf index	12. 47	2. 11	. 15	11. 48	4. 91	. 29	—. 99± . 33	3. 0

BRACHYTIC STATURE

	Mean.	σ	PE.	Mean.	σ	PE.	Difference.	D/E.
Height	8. 21	1. 92	0. 11	8. 31	2. 66	0. 25	+0. 10±0. 27	0. 4
Length fifth leaf	72. 23	10. 04	. 67	61. 30	14. 30	1. 97	—10. 93±2. 08	5. 3
Width fifth leaf	9. 42	2. 14	. 14	8. 24	2. 12	. 29	—1. 18± . 32	3. 68
Number tassel branches	23. 08	10. 10	. 66	10. 94	9. 95	1. 35	—12. 14±1. 50	8. 10
Total number leaves	24. 95	2. 04	. 18	25. 35	1. 89	. 31	+. 40± . 36	1. 11
Leaf index	14. 26	5. 92	. 40	13. 29	5. 96	. 82	—. 97± . 91	1. 06

DWARF STATURE

	Mean.	σ	PE.	Mean.	σ	PE.	Difference.	D/E.
Height	4. 80	0. 98	0. 06	5. 14	1. 03	0. 12	+0. 34±0. 13	2. 60
Length fifth leaf	45. 93	6. 17	. 36	38. 40	7. 68	. 91	—6. 53± . 98	6. 66
Width fifth leaf	11. 39	2. 22	. 13	10. 19	1. 83	. 22	—1. 20± . 26	4. 60
Number tassel branches	. 8	1. 35	. 08	. 19	. 59	. 07	—. 61± . 11	5. 54
Total number leaves	24. 52	2. 18	. 34	25. 50	1. 66	. 56	+. 98± . 65	1. 50
Leaf index	25. 47	3. 73	. 22	23. 88	. 43	. 43	—1. 59± . 48	3. 38

There seems to be no association between perfect flowered ears and liguleless leaves. The fourfold distribution for these characters is:

Normal leaves.		Liguleless leaves.		Total.
Pistillate ears.	Perfect flowered ears.	Pistillate ears.	Perfect flowered ears.	
451	158	160	40	809

The percentage of liguleless plants is 24.7 ± 1.02, of perfect flowered ears 20.8 ± 0.97, and Q (Yule's coefficient of association) $=0.167$. The expected distribution on the assumption of a 9:3:3:1 grouping would be 455.1 : 151.7 : 151.7 : 50.5, from which the observed departs by an amount which could be expected as the result of chance about 4 times in 10, ($\chi^2 = 2.94$).

The fact that all but two of the dwarf plants had staminate ear spikes, and the possibility that the absence of such spikes in these two cases may be due to the activities of the ear worm, necessitates the conclusion that this character and dwarf stature are very closely linked. It necessarily follows that the linkage relations of the staminate ear spike character in question will be identical with those of dwarf stature. But by confining the analysis of staminate ear spikes to plants of nondwarf stature, it becomes possible to measure the linkage relations of a single factor for the other heterozygous staminate ear-spike character involved in this cross.

This factor is the one not linked with brachytic stature. Confining the analysis to those plants of normal stature only, the fourfold grouping with liguleless leaves becomes:

Normal leaves.		Liguleless leaves.		Total.
Without ♂ spikes.	With ♂ spikes.	Without ♂ spikes.	With ♂ spikes.	
395	140	109	33	677

The percentage of plants with staminate ear spikes is 25.6 ± 1.13 and for liguleless leaves 21.0 ± 1.05, while $Q = 0.079 \pm 0.07$, or practically 50 per cent, of crossing over.

It may be concluded, therefore, that the gene for liguleless leaves is independent of this factor for staminate ear spikes, as well as of the corresponding factor which is linked with dwarf.

INHERITANCE OF PERICARP COLOR

The inheritance of pericarp color in this hybrid presents no new or unexpected features. The association of brachytic stature with colorless pericarp confirms previous results where these characters were found to lie from 31 to 43 units apart. The coefficient of association is found to be 0.389 ± 0.076, which for the 13 to 3 grouping is the equivalent of 38 per cent ± 2.3 crossovers.

SUMMARY

(1) There are two forms of dwarf maize in which the reduction in height is due to shortened internodes and not to a reduction in the number of internodes. One of these is known as dwarf, the other as brachytic. The variation known as dwarf also departs from the normal in characters other than stature. The most striking of these other changes are the shortened and widened leaves, the reduced number of tassel branches, and the perfect flowered ears.

(2) When the dwarf and brachytic variations are crossed, the plants of the first generation are fully as tall as normal plants from which the

brachytic variation arose. These F_1 plants are normal also with respect to the other teratological characters.

(3) In the prejugate generation three types of plants with respect to stature are found—normal, brachytic, and dwarf. These three stature groups occur in the ratio of 9:3:4, when due allowance is made for the low survival value of dwarf plants, indicating that the double recessive, a combination of dwarf and brachytic, closely resembles the dwarf parent. This indication is confirmed when the self-pollinated seed of segregated brachytic plants is grown.

(4) As was to be expected, some of the F_2 brachytic plants proved to be heterozygous for dwarf, their progenies comprising two types of plants—brachytics and dwarfs. These dwarfs represent the double recessive, being a combination of brachytic and dwarf, but are only slightly smaller than the dwarf parent of the original hybrid. They are found to have all the other characteristics of dwarf plants.

(5) From their behavior it may be concluded that dwarf and brachytic are two independent variations, both expressed in reduced stature, the genes for which are located in separate chromosomes.

(6) The analysis of the plants of the second generation with respect to all the characters which differentiate dwarf plants from the other groups shows clearly that the complex of characters associated in the dwarf variation is not inherited invariably as a unit. Thus, many of the plants of normal stature are found to have ears terminating in staminate spikes, or tassels with few or no branches, or short, broad leaves, and even a few were found with perfect flowered ears. Such behavior would indicate that the combination of characters comprising the dwarf variation formed a linkage group with very low crossover values between some of the members. On the other hand, the plants of extremely short stature, easily distinguishable from the brachytic and normal plants as dwarfs, always had other dwarf characters, such as short, broad leaves, perfect flowered ears, and few tassel branches. These dwarf segregates, like their parent, also shed little pollen, though some of the F_2 segregates seem to have improved slightly in this respect.

(7) From a consideration of the general features of such variations as anther ear, dwarf, and brachytic it is suggested that in maize, at least several and possibly all the chromosomes are identical, each having a complete assortment of genes for all the characters arranged in a similar order. Such a condition would arise through a reduplication of the chromosome number in much the same manner as that observed by Blakeslee in Datura (*1*).

(8) The recurrence of degenerative variations is discussed and the hypothesis advanced that the survival of such stocks is due to the linkage relations of deleterious characters with factors favorable for growth.

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PLATE 1

Dwarf and normal maize plants showing relative size. Compare with Plate 2 in which the same normal plant appears photographed at the same scale.

322[1]

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PLATE 2

Normal and brachytic maize plants showing relative heights.

PLATE 3

Brachytic and dwarf maize plants from which the leaves have been removed compared with a section of a plant of normal stature.


US
DA


A


B

322[4]

PLATE 4

A.—Dwarf plant from the second generation of the dwarf-brachytic hybrid, showing the type of tassel, sturdy stalk, and short, wide leaves.

B.—Brachytic, dwarf, and brachytic-dwarf maize plants. Brachytic at right, dwarf at left, and the double recessive in center. The brachytic and dwarf plants are sibs from the second generation of the dwarf-brachytic hybrid, while the double recessive plant is from the third generation of this cross, having been obtained in a progeny from a self-pollinated F_2 brachytic plant which proved to be heterozygous for dwarf. No such sharp distinction as is indicated in this picture was found in the second generation between dwarf and the double recessive. It is apparent that the double recessive form of this combination is much larger than the double recessive anther ear dwarf figured by the Emersons.

PLATE 5

Three liguleless maize plants from the second generation of the dwarf-brachytic hybrid. At left, a dwarf liguleless; center, brachytic liguleless; right, a plant of normal stature with liguleless leaves. While these plants are from the F_2, they approximate closely the heights of the two parent variations and their F_1 hybrid.

DETERMINATION OF SULPHUR COMPOUNDS IN DRY LIME-SULPHUR[1]

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INTRODUCTION

Lime-sulphur products and analogous substances, known as polysulphids, have of recent years come into prominence because of their value as insecticides and fungicides. Large quantities, especially of the lime-sulphur compound, are now manufactured. Correct and simple methods are needed for the estimation of the principal constituents, both as a safeguard to the producer and as a help to the entomologist and plant pathologist.

METHODS IN USE

Avery (*2*),[3] working with sulphur dips, modified standard methods in order to determine the total sulphur and total lime. Na_2S, resulting from the treatment of a dilute solution with NaOH, was oxidized by a large excess of medicinal H_2O_2.

Haywood (*6*, *7*), also modified standard methods in order to estimate the sulphur combined as sulphids, polysulphids, and thiosulphates. The sulphid sulphur was determined by titration with standard ammoniacal $ZnCl_2$, using $NiSO_4$ as an outside indicator. The ZnS was converted into soluble sulphid by treatment with an excess of a saturated solution of KOH. The alkali sulphid was then oxidized by means of a large excess of H_2O_2. The thiosulphate was determined in another portion of the solution, after the removal of the soluble sulphids as explained by titration with standard iodin.

Thompson and Whittier (*9*) determined the monosulphid sulphur by the addition of a slight excess of ammoniacal $CdCl_2$ to the solution of the polysulphid in the presence of KCN. The precipitated CdS was then dissolved in NaOH and the resulting Na_2S was oxidized with H_2O_2. The thiosulphate sulphur was determined in a separate portion by titration of the filtrate, from the ammoniacal $CdCl_2$ precipitation, with standard iodin. The total sulphur was estimated by precipitation with ammoniacal $CdCl_2$ and treatment of the precipitate as given under the monosulphid sulphur determination. The authors state that this method is unaffected by any free $Ca(OH)_2$ or S in solution.

The methods employed by Tartar and Bradley (*8*) consisted in the use of *N/10* ammoniacal $ZnCl_2$ with $NiSO_4$ as an outside indicator, for the determination of the monosulphid sulphur, and a titration method, *N/10* HCl being used with methyl orange as indicator, for the estimation of the polysulphid sulphur. The deposited sulphur was weighed directly, or for more accurate results, recommendation was made to bring the sulphur into solution with KOH and then to oxidize with

[1] Accepted for publication May 2, 1923.
[2] Published with the authorization of the director of the Massachusetts Agricultural Experiment Station.
[3] Reference is made by number (italic) to "Literature cited," p. 336.

H_2O_2. The originators of this method stated that the number of cubic centimeters of HCl used is the amount necessary to react with both the polysulphid and the hydroxid present, the solution always being alkaline due to the hydrolysis of the polysulphid.

Chapin (*3, 4*) originated new methods for the determination, iodometrically, of the three principal forms of sulphur, monosulphid polysulphid, and thiosulphate, the latter being the compound directly titrated in each case. His methods were based, and adjusted accordingly, on solutions containing 1.5 to 2 per cent of sulphid sulphur. Chapin stated that increasing the quantity of sample and using stronger solutions for titrations might very likely improve the accuracy in some of the results.

Harris (*5*) determined both the monosulphid and thiosulphate sulphur in diluted solutions by a double iodin titration. The amount of *N/10* iodin required to destroy the yellow color of the soluble sulphids was the monosulphid sulphur equivalent. The thiosulphate sulphur was determined by continuing the addition of iodin after the monosulphid end-point had been reached, with starch as indicator. The total sulphur was determined by oxidizing the sulphur in solution with Na_2O_2.

Averitt (*1*) estimated the monosulphid sulphur by titrating a dilute solution with *N/10* HCl, using in one case either methyl orange or methyl red as an indicator, and in another case sodium nitroprussid as an inside indicator. The thiosulphate sulphur was determined, when either of the first two indicators was used, by titrating with iodin the diluted filtrate, with or without starch, after boiling off the H_2S in solution. When sodium nitroprussid was employed, the thiosulphate sulphur was determined by a further addition of iodin until a faint coloration was produced.

RELATION OF FOREGOING METHODS TO THE CHEMISTRY OF LIME-SULPHUR

When dry lime-sulphur mixtures are subjected to analysis, it is the opinion of the writer that it is entirely unnecessary and erroneous to make a solution of high concentration and then to base quantitative results upon a diluted portion of the highly concentrated solution.

The writer believes that the weak compounds contained in dry lime-sulphur when dissolved in water exist in a complex state of equilibrium so very susceptible even to slight outside influences that these disturbing factors will seriously affect the percentage composition of the constituents if the analysis is based on a diluted solution. It is a well-known fact that polysulphids in aqueous solutions are hydrolyzed to a greater or less degree, depending upon the concentration, and to some extent upon the temperature, of the solution. The hydrolysis of calcium polysulphid might be represented by the following equation:

$$2\ CaS.\ S_x + 2\ H_2O = Ca\ (OH)_2 + Ca\ (SH)_2 + 2\ S_x.$$

The amount of sulphur deposited is of course dependent upon the concentration of the Ca $(OH)_2$.

The two points mentioned, (*a*) the action of external factors such as carbon dioxid and oxygen of the atmosphere, and (*b*) the resulting differences in the amount of sulphur in solution due to hydrolysis, should control to a large extent the preparation of the sample and the methods to be applied for the determination of the desired constituents contained in dry lime-sulphur mixtures.

Taking into consideration the above statements, it is believed to be impossible to make accurate comparisons, based on solutions, between different brands of lime-sulphur products when the products so compared are of varying composition. Therefore accurate results can be obtained only by the application of methods directly to the lime-sulphur powder and not to a diluted portion of a highly concentrated solution.

For the determination of thiosulphate sulphur all investigators accept the method used—namely, the titration of the filtrate after the removal of the free sulphur and sulphid, by the use of a standard iodin solution. The procedure followed in obtaining the thiosulphate solution, however, varies, and the results for thiosulphate sulphur are more or less influenced according to the method employed for the separation of polysulphid sulphur from the thiosulphate solution.

The determination of total sulphur is essentially alike in all cases, the total sulphur in solution being oxidized to sulphate either by the use of H_2O_2 or Na_2O_2, and determined as $BaSO_4$.

The important differences between the various methods described are in the determination of the monosulphid and the so-called polysulphid sulphur. The determination of the former by the use of an *N/10* ammoniacal solution of $ZnCl_2$ with $NiSO_4$ as an outside indicator is considered, theoretically at least, as being sound, but the difficulty experienced in obtaining pure zinc and the laborious method of procedure in the use of an outside indicator would make such a method secondary to any other method whose accuracy could be relied upon.

The inaccuracy in the estimation of monosulphid sulphur by standard iodin solution is due largely to the fact that hydrolysis of the calcium polysulphid occurs, and in consequence the iodin reacts also with the base formed, giving too high a value for the monosulphid sulphur.

The same criticisms may be applied to the iodin method as modified by Averitt. Though the sensitiveness of sodium nitroprussid is reduced when it is used with iodin as an inside indicator, this use differentiates an end-point more clearly by the production of color than does the use of iodin alone by the destruction of the color of sulphids in solution.

The end-point for the thiosulphate determination is unsatisfactory, with or without starch, because of the instability in the color developed by the addition of iodin.

The use of *N/2* HCl is preferable to *N/10* HCl for the titration of the sulphid solution, since the end-point with methyl orange is much sharper. The titration with HCl can be so conducted as to result in no appreciable action upon the thiosulphate in solution. The inaccuracy, resulting from calculating the amount of sulphid sulphur present from the number of cubic centimeters of HCl used, is believed to be due to the fact that the amount of HCl required is more nearly a measure of almost the total amount of calcium present in the solution.

The methods in which metallic salt solutions are employed for the determination of the polysulphid sulphur produce colloidal substances and the chemist is confronted with the difficulties of adsorption, slowness of filtering, and possible oxidation of sulphid.

DESCRIPTION OF PROPOSED METHODS

METHOD A

In this method the solutions used were: Hydrated sodium peroxid; barium chlorid solution, 1 to 10; *N/20* iodin; and starch.

The hydrated sodium peroxid is prepared by placing C. P. sodium peroxid under a bell jar with a separate container of water. Air is excluded to prevent the formation of sodium carbonate. The yellow color of the peroxid gradually disappears with the absorption of moisture and the hydrate ($Na_2O_28H_2O$) is formed. This hydrated product can be dissolved in water at room temperature with but slight decomposition, or in cool water with practically no decomposition at all. The solution of sodium peroxid may be brought to the temperature of the steam bath with but slight loss of oxygen, and if used at this higher temperature increases the rate of oxidation of the hydrogen sulphid.

PROCEDURE

Sufficient hydrated sodium peroxid and 100 cc. of distilled water are added to flasks No. 1 and No. 2 (fig. 1) and the flasks connected to the apparatus; 0.5 gm. of dry lime-sulphur is transferred to the reaction

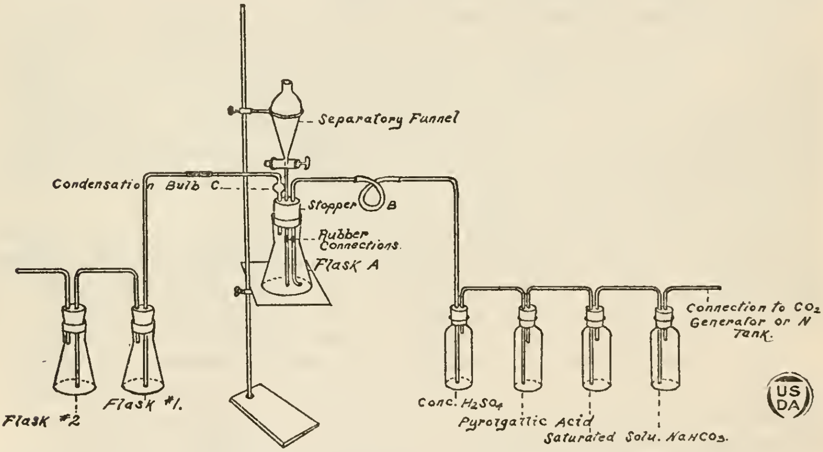

FIG. 1.—Apparatus for the direct separation of the three forms of sulphur—namely, monosulphid, thiosulphate and polysulphid sulphur (plus any free sulphur). The hydrogen sulphid or monosulphid sulphur will be in solution in flask No. 1, thiosulphate sulphur in solution in flask A, and the precipitated or residual sulphur in flask A will consist of the polysulphid sulphur plus any free sulphur.

flask, A. Carbon dioxid (generated by the action of hydrochloric acid upon marble in a Kipp generator), freed from all traces of hydrochloric acid and moisture by passage through a saturated solution of sodium bicarbonate and then through a solution of concentrated sulphuric acid, is forced through the apparatus to replace all air, and without shutting off the current of carbon dioxid, 50 to 60 cc. of distilled water are added to flask A by means of the separatory funnel. The reaction flask is shaken continuously for from three to five minutes, after which time the agitation is carried out intermittently until all the hydrogen sulphid has been expelled and absorbed in the alkaline solutions contained in flasks No. 1 and No. 2.

The contents of flask A are filtered, after the complete expulsion of all traces of hydrogen sulphid, through a platinum Gooch crucible with a thin asbestos pad, and the residue in the crucible is well washed and the filtrate made up to a volume of 200 cc. and titrated with *N/20* iodin with starch solution as indicator. The iodin titration determines the thiosulphate sulphur.

Any sulphur adhering to the two pieces of glass tubing which dipped into the solution contained in flask A, is added to the bulk of sulphur in the crucible, and the contents of the crucible are treated with 1 to 10 hydrochloric acid to dissolve the precipitated calcium carbonate. The residue is well washed, after which the crucible and contents are dried at 100° C. for one hour, weighed, ignited, and again weighed. The difference in weight represents the sulphur present in the polysulphid form together with any free sulphur.

The sulphur evolved as hydrogen sulphid, and contained in the solution in flasks No. 1 and No. 2[4] as sodium sulphate, is transferred to 500 cc. graduated flasks and heated upon the steam bath with the flasks stoppered until complete oxidation is assured. The solution is cooled and a few drops of methyl orange are added, followed by hydrochloric acid until the solution is slightly acid. It is then boiled to destroy the hydrogen peroxid and to drive out all carbon dioxid, cooled, and made to volume.

To 50 cc. portions of the solution ½ cc. of 1 to 1 HCl is added, and the sulphate determination is carried out in the usual manner.

METHOD B

In this method the solutions used are: Hydrated sodium peroxid; approximate *N/2* HCl; barium chlorid solution 1 to 10; *N/20* iodin; and starch.

PROCEDURE

Five-tenths gm. dry lime-sulphur is placed in a small Erlenmeyer flask, 50 to 60 cc. of recently boiled and cooled distilled water added, and the solution titrated, with vigorous agitation of the contents of the flask, with approximate *N/2* HCl, and 3 to 4 drops of methyl orange indicator introduced just previous to the end-point of the reaction. The addition of HCl is continued until the pink color developed persists in the solution after the flask has stood for two or three minutes. The amount of HCl used determines the total basicity of the solution.

The prepared Na_2O_2 solution is then added to flasks No. 1 and No. 2 as in Method A.

Another 0.5 gm. sample of the dry lime-sulphur mixture is transferred to the reaction flask, A, and oxygen-free N passed through the apparatus. To the separatory funnel is added the same quantity of approximate *N/2* HCl which was required to neutralize the basic constituents contained in 0.5 gm. of dry lime-sulphur. The sides of the funnel are rinsed with 50 cc. of distilled water and the diluted acid solution is carefully admitted to the reaction flask without interruption of the slow passage of N. The funnel is again rinsed with a small amount of water, a few drops of methyl orange added, and the solution run carefully into the reaction flask.

The solution contained in flask A should develop a pink color provided all the acid has been removed from the separatory funnel and the separated sulphur has not completely absorbed the indicator.

The passage of N through the apparatus is continued until all traces of H_2S have been expelled from flask A. From this point the procedure is similar to that given under Method A.

[4] Flask No. 2 is used as a precaution and will be found to contain no sulphur, provided the gas is not forced through the apparatus at too rapid a rate.

ADVANTAGES OF PROPOSED METHODS

The advantages derived from the use of either of the two methods are based on accuracy, simplicity of reactions, and the employment of but few and simple operations.

The claim for accuracy is based on the following reasons:

1. Both methods are based directly on the use of dry lime-sulphur and not upon prepared solutions. The methods are applicable, however, to solutions of lime-sulphur.
2. All results are obtained on the one sample throughout the determination.
3. The liberation of sulphur, equivalent to the monosulphid sulphur of the metal, is made complete by the action of dilute acids upon the polysulphid in solution.
4. The evolution of the hydrogen sulphid and the complete removal by aid of a gas allows clear and complete separation of the three forms of sulphur.
5. The determination of monosulphid sulphur, when present in large quantities, can not be more accurately made than by the evolution method; and the absorption, oxidation, and weighing as $BaSO_4$ is as accurate a method for the final determination as any method yet devised.
6. The thiosulphate sulphur is determined by the usual and exact method of oxidation by means of iodin.
7. The sulphur remaining after the complete removal of the monosulphid sulphur is readily separated from the thiosulphate solution by simple filtration and is determined by weight.

The application of these methods is simple because:

1. Complexity in reactions and processes is eliminated by the use of one process for the complete separation of the three forms of sulphur.
2. The time required for the determination is reasonably short, and the process of expelling the hydrogen sulphid from solution does not require the constant attention of the operator.
3. The use of a solution, in connection with hydrogen sulphid, which answers the purpose of an absorbing and oxidizing agent saves time and promotes accuracy.

DISCUSSION OF METHODS

These methods do not include any procedures dealing with the detection or estimation of any of the possible and theoretical sulphur compounds, stated by a few investigators as existing in solution as traces, but are concerned wholly with the determination of the principal constituents contained in the commercial lime-sulphur products, which are considered to give to lime-sulphur solutions their insecticidal and fungicidal value—namely, monosulphide and residual and thiosulphate sulphur.

Both methods are based upon the fact that the acids used are ionized to a greater extent than the hydrosulphuric, and therefore have the power of decomposing completely the polysulphids of calcium, with liberation of hydrogen sulphid and deposition of sulphur, according to the following equations:

1. $CaS.S_x + H_2O + CO_2 = CaCO_3 + H_2S + S_x$.[5]
2. $CaS.S_x + 2\ HCl = CaCl_2 + H_2S + S_x$.

[5] Evidence that carbonic acid completely decomposes the sulphids in solution can be obtained by testing the solution at the end of the experiment with sodium nitroprussid.

The method as represented by the first equation might be designated as the carbonic acid method, and is called method A; the other, as represented by the second equation, might be designated as the hydrochloric-acid method, and is referred to as method B.

No attempt is made by the use of either of the proposed methods to calculate the amount of polysulphid sulphur present in the dry lime-sulphur mixture, because of the uncertainty as to just what proportion of the residual sulphur (contained in the reaction flask) can be attributed to polysulphid sulphur and what amount to soluble free sulphur.

The same uncertainty is apparent upon application of methods based on aqueous solutions of polysulphids. If the lime-sulphur mixture contained free sulphur, the addition of water resulting in the solution of the lime-sulphur compounds would naturally precipitate some if not all of the free sulphur in solution, the quantity deposited depending upon the dilution. On the other hand, the hydrolysis of polysulphids in aqueous solutions results also in the deposition of sulphur, the amount depending upon the dilution and temperature of the solution and the concentration of calcium hydroxid formed. In either case, whether methods are applied to the dry lime-sulphur products or to solutions of the same, it is quite impossible to differentiate accurately between true polysulphid of sulphur and free sulphur in solution.

The true polysulphid sulphur might be estimated with some accuracy, provided there existed a method for determining different amounts of free sulphur in the presence of water soluble polysulphids.

The fact is recognized by the writer that the total amount of soluble sulphur can be estimated only by analysis of the solutions of lime-sulphur mixtures. But in such analyses the solution used should be of the same concentration as that used for spray or as that recommended by the manufacturer of the product.

EXPERIMENTAL TESTS WITH METHODS A AND B

The samples of dry lime-sulphur used in the following experiments were received from the same manufacturers upon two different occasions. The results given in Table I are from a sample received and analyzed in 1919. All other results are from a sample received and analyzed in 1921.

The methods taken for comparison were the HCl and iodin methods recommended by Averitt. They were chosen because of the advantages claimed by the originators and others. There can be no criticism in regard to the quickness and ease of manipulation, but the inaccuracy of results is clearly apparent from the following work.

It was thought that numerous comparisons were unnecessary, provided it could be proved that the use of H_2CO_3 results in a quantitative separation of the monosulphid sulphur from the thiosulphate sulphur and that the use of an excess of CO_2 has no appreciable effect upon the calcium thiosulphate in solution. The experimental work as given in the following pages is believed to substantiate the accuracy of the proposed methods.

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TABLE I.—*Comparison between carbonic method and the hydrochloric and iodin methods recommended by Averitt for determining soluble sulphur found in sample of dry lime-sulphur*

Form of sulphur determined.	Methods recommended by Averitt.		Carbonic method A.
	Hydrochloric.	Iodin.	
	Per cent.	*Per cent.*	*Per cent.*
1. Monosulphid [1]	13.21	11.25	8.86
2. Thiosulphate	1.56	1.40	1.80
3. Residual	50.84	48.03	51.02
Total	65.61	60.68	61.68

[1] The differences in the determination of monosulphid sulphur as shown in this table are very marked, and similar variations were observed when the methods were applied to other samples.

TABLE II.—*Results obtained by use of the carbonic method A*

Experiment No.	Dry lime-sulphur.	Length of time of experiment.	Residual sulphur.	Thiosulphate sulphur.	Monosulphid sulphur.	Absorption solution.
	Gm.	*Hours.*	*Per cent.*	*Per cent.*	*Per cent.*	
1	0.25	1⅓	50.64	2.75	8.32	Na_2O_2.
2	.25	1¾	50.60	2.73	8.35	Do.
3	.25	1⅔	50.68	2.75	8.33	Do.
4	.25	1⅔	50.64	2.75	8.40	Do.
5	.25	1¾	50.68	2.75	8.34	Do.
Average			50.65	2.75	8.35	
6	.50	4¼	51.00	2.62	8.41	Do.
7	.25	1	50.64	3.01	(*a*)	Ammoniacal H_2O_2.
8	.25	2	50.64	3.01		Do.
9	.50	2	50.64	2.56		Do.
10	.50	2	50.64	2.56		Do.
Average			50.64	2.79		
11	1.00	3½	50.55	2.58		Do.
12	.25	17½	50.76	2.73		Do.
Average			50.66	2.66		

[a] Monosulphid sulphur was not determined for the reason that the H_2O_2 solution used was later found to contain sulphate.

TABLE III.—*Results obtained by use of the carbonic method A*—Continued

Experiment No.	Dry lime-sulphur.	Length of time of experiment.	Residual sulphur.	Thiosulphate sulphur.	Monosulphid sulphur.	Absorption solution.
	Gm.	*Hours.*	*Per cent.*	*Per cent.*	*Per cent.*	
1	0.5	5	50.63	2.64	8.40	3.6 normal NaOH.
2	.5	5	50.64	2.61	8.35	4.7 normal NaOH.
3	.5	5	50.60	2.58	8.35	NH_4OH.
Average			50.62	2.61	8.37	
4	.5	4	50.56	2.68	{[a] 7.91 [b] 8.46	Weak standard I_2.
5	.5	3½	50.60	3.00	{[a] 8.33 [b] 8.32	Weak standard I_2.

[a] By titration with standard $Na_2S_2O_3$ solution. [b] By weight of precipitated sulphur.

The data given in the Tables II and III show that the results secured for the different forms of sulphur—residual, thiosulphate, and monosulphid—are quite concordant, whether the length of time of the experiment was one hour or a longer period. The use of hydrated Na_2O_2, 3.6 and 4.7 normal NaOH, and NH_4OH also served satisfactorily as absorption solutions. Further experiments with iodin were not as satisfactory.

Further evidence of the completeness of the action of carbonic acid is borne out by a comparison with the results obtained by the use of hydrochloric acid. Five-tenths gm. of dry lime-sulphur from the same sample used in establishing the proposed methods was transferred to the reaction flask, A, dry and oxygen-free CO_2 passed through the apparatus, a sufficient quantity of distilled water added to cover the ends of the inlet tubes, and, finally, 8.36 cc. of *N/2* HCl, an amount previously found necessary to neutralize the basicity of the solution, were slowly added to the sulphid in solution. All traces of acid adhering to the separatory funnel were removed by rinsing with water containing a few drops of methyl orange. That the quantity of acid added was sufficient was indicated by a faint pink coloration of the solution. The contents of the flask were agitated during and after the admission of the acid, and CO_2 was passed through the solution for a period of 4½ hours.

The results obtained upon analysis of the three forms of sulphur are as follows:

Residual sulphur	50.96 per cent.
Thiosulphate sulphur	2.48 per cent.
Monosulphid sulphur	8.47 per cent.

Another experiment was made, using the same weight of dry lime-sulphur from the same sample but increasing the amount of $N/_2$ HCl to a large excess over that actually required and substituting pure nitrogen gas in place of CO_2. The solution in this case was brought to the boiling point near the end of the operation. The results obtained are as follows:

Residual sulphur	51.56 per cent.
Monosulphid sulphur	8.35 per cent.

The percentage results obtained for monosulphid sulphur in these two experiments are in close agreement with the results obtained by the use of the carbonic-acid method. The slight increase in the percentage of residual sulphur is due to the action of HCl upon the thiosulphate in solution which causes a precipitation of sulphur.

DETERMINATION OF MONOSULPHID AND THIOSULPHATE SULPHUR AFTER A PREVIOUS SEPARATION OF SULPHID FROM THIOSULPHATE

The accuracy of the monosulphid and thiosulphate sulphur results as determined by the carbonic-acid method was again established by the following experimental work in which the same sample of dry lime-sulphur was used.

Five-tenths gm. of dry lime-sulphur was transferred to a small Erlenmeyer flask, to which was quickly added an excess of freshly prepared and well-washed $ZnCO_3$ held in water suspension. The flask was immediately stoppered and shaken vigorously, and the shaking repeated at intervals during a period of one hour, after which the ZnS mixed with free S and excess $ZnCO_3$ was filtered and washed free from the thiosulphate in solution. The thiosulphate sulphur, which was found to equal 2.65 per cent, was determined by titrating the filtrate with *N/20* iodin.

The ZnS mixture upon the filter paper was transferred, by washing, into the reaction flask, A, air was removed from the apparatus by the use of N, HCl in excess added to decompose the carbonate and sulphid, and the gases evolved were expelled by the further use of N. The monosulphid sulphur was determined in the usual way, by oxidation to sulphate and weighing as $BaSO_4$, and was found to be equivalent to 8.41 per cent.

The employment of $ZnCO_3$ in water suspension as a means of separation of the sulphid from the thiosulphate was chosen because of the rapid rate of reaction between the $ZnCO_3$ and the soluble sulphid, the readiness with which filtration could be accomplished, and, furthermore, for the reason that constant results for thiosulphate sulphur were found not to be dependent upon the length of time of contact between the $ZnCO_3$ and the soluble sulphur compounds, as was the case when $CdCO_3$ was used.

ACTION OF CARBONIC ACID UPON CALCIUM POLYSULPHID

The power of carbonic acid to decompose water soluble sulphids can be easily demonstrated by the passage of carbon dioxid into an aqueous solution of lime-sulphur compounds. The completeness of the reaction can be positively verified by testing the solution in which the sulphid was dissolved by the introduction of sodium nitroprussid, after the carbonic acid has been allowed to react for a sufficient length of time.

The time necessary for the complete decomposition of polysulphid is very short, as indicated by the disappearance of the yellow color of the polysulphid in solution. Complete decomposition was effected, repeatedly, within from two to three minutes, when 0.25 gm. charges were used. In the case of larger charges the time was naturally lengthened, requiring from three to four minutes with charges as large as 0.5 gm. The time is based upon the passage of carbon dioxid at a moderate rate. In Table II are tabulated results expressing the quantitative reaction between carbonic acid and lime-sulphur compounds.

EFFECT OF CARBONIC ACID UPON THIOSULPHATE

Investigators of lime-sulphur solutions who have made use of HCl in the process of analysis claim that the neutralization of the solution can be brought about by the careful addition of *N/20* acid, in the presence of methyl orange or methyl red as indicator, without its reacting upon the thiosulphate. If a highly ionized acid, such as HCl, can be so used without destroying any of the thiosulphate, then the chances of a destructive action occurring with the use of an acid which is ionized to a much less degree should be, theoretically at least, even less.

In corroboration of this reasoning, experiments were conducted for the purpose of ascertaining whether or not the action of carbonic acid for different lengths of time had any appreciable effect upon the thiosulphate. The results given in Table II indicate that the weakly ionized acid does not react with the thiosulphate. The occurrence of the observed slight differences in the amounts of thiosulphate sulphur may be due to slight variations in the homogeneity of the lime-sulphur mixtures, or to slight changes in the chemical constituents resulting from repeated openings of the bottle containing the lime-sulphur powder. The perfect agreement in results between a one-plus hour, and the 17½-hour period might be assumed as conclusive proof that the concentration of hydrogen-ions resulting from the solution of H_2CO_3 is without effect upon a solution of calcium thiosulphate.

HYDROCHLORIC ACID AS A MEASURE OF THE BASICITY OF THE SULPHID SOLUTION

That the number of cubic centimeters of HCl used in the direct acid titration method can not be taken as an index of the quantity of monosulphid sulphur present in lime-sulphur mixtures, but may be taken as a measure of the basicity of the solution, is borne out by the following experiments.

Five-tenths gm. charge of dry lime-sulphur was analyzed by the carbonic acid method; CO_2 was passed through the solution for four hours, the solution brought to boiling, and the CO_2 passed through the solution for another hour. The contents of the reaction flask, A, containing free S, $CaCO_3$, $Ca(HCO_3)_2$, and CaS_2O_3, were then titrated with *N/2* HCl, using methyl orange as indicator. It was found that 8.23 cc. of acid were required to neutralize the solution.

Upon application of the direct acid titration method (Averitt) it was found that 8.26 cc. of *N/2* HCl were necessary to carry the reaction to the end point of methyl orange. The number of cubic centimeters of acid used according to the method is the equivalent of the monosulphid sulphur present. Therefore the amount of the latter, based on a 0.5 gm. sample, should equal 13.24 per cent.

A comparison of these two simple tests shows that in one case 8.26 cc. of *N/2* HCl were used in neutralizing the polysulphid solution, while in the other case almost an equal amount of HCl was required to neutralize the basic salts present, after the removal of the monosulphid sulphur.

The only possible deduction is that the HCl reacts with not only the calcium sulphid but other calcium compounds as well, and consequently the amount of HCl required can not be used as a direct estimation of the monosulphid sulphur present.

That 13.24 per cent for monosulphid sulphur is much too high is again made apparent by completing the former experiment in the usual way. The results obtained for the three forms of sulphur are as follows:

Residual sulphur	50. 64 per cent.
Thiosulphate sulphur	2. 61 per cent.
Monosulphid sulphur	8. 35 per cent.

ABSORPTION SOLUTIONS

Different absorptive solutions for the evolved H_2S were substituted in place of the hydrated sodium peroxid. Among the solutions tried were the following: Ammoniacal H_2O_2, ammoniacal $ZnCl_2$, ammoniacal $CdCl_2$, acetic acid solution of $Zn(C_2H_3O_2)_2$, dilute standard solution of iodin (with a protective flask containing a standard solution of $Na_2S_2O_3$), 3 to 4 N KOH and NaOH solutions.

With the exception of the alkali solutions, all these proved unsatisfactory for one reason or another. Solutions of hydrogen peroxid invariably contain sulphates in greater or less quantity and consequently necessitate the employment of exact measured amounts and require that blank tests be made upon each lot of peroxid solution used. The use of zinc and cadmium solutions, neutral or alkaline, gives precipitates of metallic sulphids which are of a decidedly colloid nature. The difficulties attending filtration and washing are well known. Oxidation of the sulphids during the operation is a constant source of error tending toward low results. The sulphid can not be weighed directly with accuracy because of occlusion, and, therefore, must necessarily be changed into some other form in order that the estimation of the sulphur content may be made.

Iodin solutions used as an absorbent in the determination of the monosulphid sulphur must be very dilute as the tendency for the precipitated sulphur to oxidize increases with increase in concentration. Occlusion of iodin by the sulphur and the volatilization of iodin, due to the passage of the gas through the solution, were found also to increase with increased concentration of the iodin solution. Good results were obtained, however, in a few determinations (Table III) by the use of a weak iodin solution *N/20* or less, containing an appreciable quantity of acetic acid and a large amount of potassium iodid. Starch solution in the train following the iodin solution showed no volatilization of iodin. The determination of sulphur by weighing was in agreement with the estimation made by titrating the excess of iodin. On the average the use of an iodin solution as a means of determining the monosulphid sulphur was found unreliable.

There is no gain in accuracy in the use of pure alkali solutions as absorptive agents over that obtained by the employment of a hydrated sodium peroxid solution (Tables II and III). The operation is somewhat lengthened for the reason that after the absorption the sulphur must be oxidized. The oxidation is best accomplished by the use of sodium peroxid which can easily be obtained free from sulphates.

In the opinion of the writer it is advantageous, as well as accurate, to make use of a solution which answers the purpose of an absorptive and oxidizing solution at the same time.

RESULTS FOR TOTAL SULPHUR

COMPARISON BETWEEN THE CARBONIC ACID METHOD AND METHOD OF DIRECT OXIDATION

The total sulphur was determined by direct oxidation with Na_2O_2. To 0.25 or 0.5 gm. of dry lime-sulphur were added 50 to 100 cc. of freshly boiled and cooled distilled water. Hydrated Na_2O_2, or the dehydrated form, was then added in small amounts until the oxidation was completed, the flask being kept stoppered. The procedure which followed was the same as that used in determining the monosulphid sulphur. A comparison of the total sulphur determined in this way with that obtained by the summation of the three forms as determined by the carbonic acid method (average of results given in Tables II and III) is given below.

			Total sulphur.
Direct oxidation			61.62 per cent.
H_2CO_3 method	Residual sulphur	50.64 per cent.	
	Thiosulphate sulphur	2.70 per cent.	
	Monosulphid sulphur	8.36 per cent.	
			61.70 per cent.

HYDROLYTIC ACTION RESULTING FROM AQUEOUS SOLUTIONS OF LIME-SULPHUR COMPOUNDS.

Figure 2 is illustrative of the hydrolysis of lime-sulphur compounds in aqueous solutions and is therefore indicative of errors which may arise when the analysis of dry lime-sulphur is based on diluted portions of concentrated aqueous solutions.

The experiments were conducted as follows:

The different amounts of dry lime-sulphur were taken and transferred to a 500 cc. Erlenmeyer flask [6] and freshly boiled distilled water cooled

[6] The same flask was used for each experiment.

to 20° C. was added until a volume of 535 cc. resulted. The flask was immediately corked and sealed with paraffin, leaving but a very small air space. The flask was then allowed to stand at room temperature with shaking for 48 hours, after which the deposited sulphur was rapidly transferred, by forced filtration, to a platinum Gooch crucible with a thin asbestos pad, thoroughly washed, dried for one hour at 100° C., and the crucible and contents weighed, ignited, and reweighed to obtain the amount of sulphur.

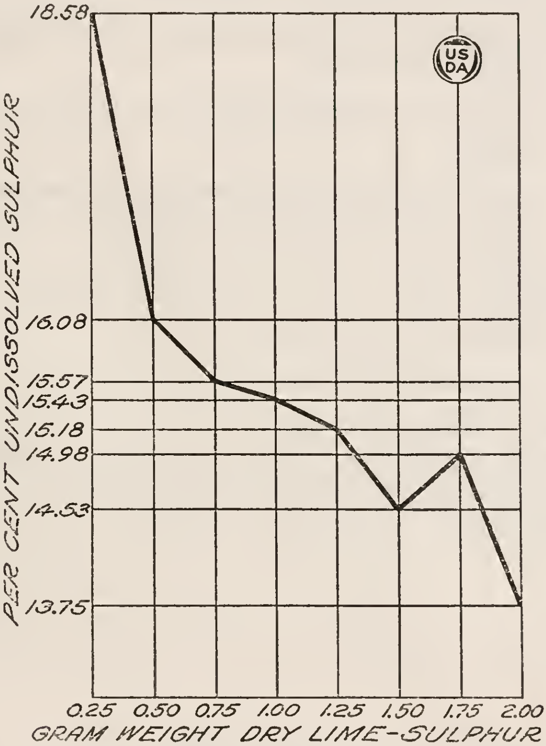

FIG. 2.—Diagram showing variations in percentage of undissolved sulphur due to chemical equilibrium resulting from the hydrolysis of lime-sulphur compounds in aqueous solutions.

SUMMARY

(1) The three forms of sulphur, monosulphid, residual, and thiosulphate sulphur, contained in lime-sulphur powders may be easily and accurately determined by the passage of CO_2 through a solution of the polysulphid. The accuracy of the method is based upon the fact that CO_2 separates quantitatively the monosulphid sulphur from the thiosulphate and residual sulphur. The use of this method allows for a complete separation of the residual from the thiosulphate sulphur, thus enabling accurate estimation of both.

(2) The method is entirely free from any laborious process of filtering and uncertainty regarding end-points in the use of indicators.

(3) The application of this method eliminates all errors due to hydrolysis of lime-sulphur compounds in aqueous solutions and also the reacting influences of CO_2 and O of the atmosphere.

(4) The use of hydrated Na_2O_2 for the absorption of H_2S has a twofold advantage. The alkalinity of the solution acts as a binding agent while the 20 per cent of available O acts in the capacity of a powerful oxidizer.

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THE TWINNING AND MONEMBRYONIC DEVELOPMENT OF PLATYGASTER HIEMALIS, A PARASITE OF THE HESSIAN FLY[1]

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INTRODUCTION

Those who are familiar with the polyembryonic method of development of some insects have always thought it desirable that a study be made of the development of a species which produces but a small number of individuals from a single egg. In the four species of insects in which the polyembryonic method of development has been previously described in greater or less detail, the number of individuals produced from a single egg has been 150 to about 1,700. These species represent a highly specialized type of polyembryony, whereas a species in which one egg produced only two or four individuals would be expected to illustrate a more simple type of polyembryony.

Platygaster hiemalis Forbes, a hymenopterous parasite of the Hessian fly,[3] is a species which develops both monembryonically and polyembryonically. This parasite deposits a group of from 5 to 8 eggs in the egg of its host, and an average of 6.31 individuals are developed from these eggs. Some of the eggs of the group fail to develop at all, others of the group develop twins, while still other eggs of the same group give rise to single individuals. The objects of this paper will be to describe the twinning and monembryonic development of this insect, to show why some of the eggs fail to develop, and to point out the relation between the more simple type of polyembryony here called twinning and monembryony.[4]

RELATION OF PARASITE TO HOST

Platygaster hiemalis is single-brooded, although its host, the Hessian fly, a serious wheat pest, is double-brooded throughout most of the winter-wheat region of the United States. The adults of *P. hiemalis* emerge from their cocoons in the host puparia (Pl. 1, D) during the fall of the year, at which time the Hessian flies of one generation also emerge. The parasite deposits its eggs in the eggs of the fly which have meanwhile been deposited on the wheat plants.

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[1] Accepted for publication Aug. 11, 1923.

[2] The authors desire to express their appreciation of the interest shown by Dr. L. O. Howard and W. R. Walton in the studies reported in this paper.

[3] *Phytophaga destructor* Say.

[4] A detailed account dealing with the bionomics of this parasite and its economic importance as an enemy of the Hessian fly is being prepared by the junior author.

The early development of the parasite takes place in the host egg and in the young host larva during fall (Pl. 1, C, E), the winter being passed in the embryonic stage within a well developed host larva, which remains on the wheat plant. In spring the Hessian fly larvæ, if unparasitized, soon produce adult flies. Meanwhile, the parasites in parasitized host larvæ complete the embryonic stage of development, and remain in this condition in the fully grown host larvæ until early summer. During July the newly developed parasite larvæ mature by feeding upon the body content of the host. The parasite larvæ then pupate in individual cocoons made within the host puparium, the adult parasites finally emerging from their cocoons in the fall.

DEVELOPMENT OF THE EGG TO CLEAVAGE

The precleavage development of the egg of but one species of the Platygastridae (*Platygaster dryomyiae* Silv.) which develops by the monembryonic method has been described by Silvestri (*11*).[5] This phase of the development of polyembryonic Hymenoptera has been demonstrated by Silvestri (*9*) and Patterson (*5*) in *Copidosoma truncatellum* and by Leiby (*2*) in *Copidosoma gelechiae.* In all cases it has been shown (1) that two maturations of the oocyte nucleus occur, (2) that the two resulting polar bodies are not thrown off (as they usually are in monembryonically developing eggs) but are retained in the anterior or polar region of the egg, (3) that the polar region is differentiated from the posterior or embryonic region, and forms together with the polar bodies a nucleated membrane which encompasses each embryo in the course of its development, and (4) that the fertilized or unfertilized cleavage nucleus divides and is destined eventually to give rise to the embryos. The processes by which the precleavage stages of these eggs take place differ only slightly, but the later stages of development of the demonstrated species are known to differ very markedly.

THE NEWLY DEPOSITED EGG

The eggs of *Platygaster hiemalis* are regularly deposited in groups of from 4 to 8, at each oviposition in the host egg. Hence they are frequently found in contact and side by side (Pl. 1, A), or very close to each other. It, therefore, is not difficult to ascertain whether a host egg has been oviposited in more than one time, or how many eggs are deposited at a single oviposition.

The newly deposited egg (Pl. 1, E) is ovoid in shape, usually bluntly rounded at the posterior end, and somewhat pointed at the anterior end. Sectioned eggs measure 15μ in length and 6μ in width, or 5μ in length and 2μ in width less than freshly deposited unfixed eggs. The cytoplasm of the egg is densely and finely granular, homogeneous throughout, and without oil spherules or vacuoles. The presence of a weakly defined membrane or chorion becomes evident in eggs that shrink considerably following some fixations.

The egg nucleus is spherical and measures 2.5μ in diameter. It is regularly found in or very near the center of the egg. When sectioned the nucleus of the newly deposited egg exhibits its chromosomes scattered over a reticulum.

[5] Reference is made by number (italic) to "Literature cited," p. 349.

If the egg is inseminated the sperm can be seen usually in a curved or arched position (Pl. 1, E; Pl. 2, A–C), and frequently extending more than two-thirds the length of the egg. The body part and tail of the sperm measure 2.7μ and 7.3μ, respectively. Never more than one sperm has been found in one egg. It is quite evident that not all of the eggs deposited at one time by a fertilized parasite contain sperms. This point has been studied thoroughly, careful examinations of groups of eggs showing that about one-third of the eggs are not inseminated. For example, only 5 of the group of 8 eggs shown at F on Plate 1 contain sperms.

There is no evidence whatever of the presence of a nucleolus or so-called germ-cell determinant. Nor did Silvestri find this nuclear body in *Platygaster dryomyiae*, although it has been recorded as present in the eggs of polyembryonic Hymenoptera previously described.

MATURATION

Both fertilized and unfertilized eggs maturate and develop adult parasites, the fertilized eggs presumably giving rise to females and the unfertilized eggs developing males; although on this point no conclusive biological or cytological evidence is available. The process of maturation is identical in fertilized and unfertilized eggs.

During the first hour after oviposition the oocyte nucleus (Pl. 1, E; Pl. 2, A) increases slightly in size and becomes clearer, whereupon the chromosomes are seen scattered over a reticulum. The nucleus then becomes more concentrated (Pl. 2, B) and is found immersed in a semi-clear area of the oocyte cytoplasm. During this time its chromatin appears as a condensed mass of fine granules. Two hours after oviposition the nucleus again expands slightly (Pl. 2, C) and chromatin bodies are to be seen supported at the nodes of the reticulum. At four hours the nucleus is found at prophase, in the act of forming a spindle, or at anaphase (Pl. 2, D–F). The spindle is always arranged longitudinally in the egg. At the close of the first maturation the first polar body is always found at the anterior pole while the oocyte nucleus of the second order is found near the center of the egg (Pl. 2, G).

Previous to the second maturation, which takes place at about the seventh hour, the first polar body becomes concentrated and appears as an irregular homogeneous mass of chromatin that measures about 1μ in diameter. Between the fourth and the seventh hours, the oocyte nucleus of the second order undergoes changes similar to those described for the nucleus previous to the first maturation; and at the end of the seventh hour the nucleus is found at prophase (Pl. 2, J). The spindle which is then formed is again disposed longitudinally in the anterior half of the egg (Pl. 2, L, M).

After the second division, the second polar body is seen in the anterior end, near or adjacent to the first polar body; while the oocyte nucleus of the third order (later the female pronucleus) remains in the center of the egg until about the ninth hour, after which it may be found almost anywhere in the posterior half of the egg. From the eighth to the sixteenth hour the female pronucleus is apparently in a resting stage, although during this interval it increases in diameter from $1.8\ \mu$ to $3.5\ \mu$.

FERTILIZATION

Prior to first maturation of the oocyte nucleus, the sperm is found in any part of the inseminated egg; but shortly afterward it assumes the appearance of a somewhat elongate or crescentic nuclear body (Pl. 2, A–I). It is regularly found thereafter in the posterior half of the egg.

After the second maturation the male nucleus becomes spherical, stains deeply, and measures 1.5 μ in diameter (Pl. 2, K–P). At the eleventh hour the male pronucleus expands until it measures 2 μ in diameter. At about the twelfth hour the male and female pronuclei are found regularly close to each other (Pl. 2, R–T) in the posterior part of the egg. Fusion of these two nuclei (Pl. 2, U) is effected at about the sixteenth hour. The egg now contains a polar nucleus in the anterior region and a single cleavage nucleus located in the posterior region (Pl. 2, W).

ORIGIN OF THE PARANUCLEUS

During the development of the embryos rounded or crescentic nuclear masses (Pl. 3, A–F) are found in the nutritive membrane (trophamnion) which surrounds the germs and blastulas. These were termed paranuclear masses by Marchal (*3*) in a species of Encyrtus that develops by polyembryony. Silvestri (*9*, *10*, *11*), Patterson (*8*), and Leiby (*2*) have shown in other polyembryonic Hymenoptera that the paranuclear masses have their origin in the polar bodies. Paranuclear masses having a similar nutritive function are not confined to the polyembryonic Hymenoptera; for they have also been demonstrated by Marchal (*4*) in the monembryonically developing species of Synopeas, Trichasis, and *Platygaster ornatus*.

The two polar bodies developed in the course of maturation in *Platygaster hiemalis* also give rise to a polar nucleus or paranuclear mass. At the close of the eighth hour they lie close to each other in the anterior end of the egg (Pl. 2, N), where they are recognized as compact, darkly staining masses of chromatin measuring approximately 0.7 μ and 1.0 μ in diameter, respectively.

The first polar body, unlike those in all other polyembryonic Hymenoptera previously described, does not divide during second maturation. Instead, the two polar bodies fuse to form a single polar nucleus (Pl. 2, O–Q) at about the tenth hour. This behavior of the polar bodies differs from that shown by Silvestri (*11*), in *Platygaster dryomyiae*, in which he found that the first polar body divides, and that the anterior half of the divided first polar body forms one polar nucleus, while the posterior half of the first polar body and the second polar body fuse to form a second polar nucleus.

Between the tenth and twelfth hours the polar nucleus elaborates until it fills most of the anterior region of the egg and measures about 4.5 μ in diameter (Pl. 2, R). It remains a single more or less spherical nucleus (Pl. 2, S–V) until just before the division of the cleavage nucleus, when it divides amitotically to form two subequal paranuclear masses (Pl. 3, A).

DIFFERENTIATION OF EMBRYONIC REGION AND FORMATION OF TROPHAMNION

About the twenty-fourth hour two regions are recognized in the egg, when the central part of its posterior half becomes distinctly differentiated

from the remainder of the egg (Pl. 2, X). This differentiated region contains the cleavage nucleus, and is called the embryonic region, since it later gives rise to the embryos. The remainder of the egg containing the polar nucleus constitutes the polar region.

Between the first and second days the group of from 5 to 8 eggs deposited by the parasite in the host becomes somewhat dispersed throughout the developing host if they were deposited in a host egg. If the eggs were deposited in an embryo well advanced in development at the time of oviposition or in a recently hatched host larva, they become scattered in the body cavity of the host at an earlier hour. In any event the eggs begin to increase in size soon after the first day, when they measure 18.2 μ in length and 7.8 μ in width. Since the eggs increase in size, the egg stage may be regarded as past and henceforth the eggs may be denoted as parasite bodies.

During the dispersal of the parasite bodies in the cavity of the host they become lodged against the host tissues, such as the salivary glands or fat tissues, whereupon portions of these tissues soon encompass the parasite bodies. Occasionally two parasite bodies may be surrounded by the same bit of host tissue, but both parasite bodies will usually continue to develop independently of each other. After each parasite body is surrounded (at least partially) by host tissue, an elaboration of the paranuclear masses in the polar region can immediately be observed. This elaboration is obviously due to the absorption of host tissues by the parasite body through its polar region. The polar region, including that part which surrounds the embryonic region, is therefore properly known as the trophamnion.

CLEAVAGE TO BLASTULA STAGES

The development of the embryos, from cleavage of the segmentation nucleus to formation of the blastulas, takes place for the most part in the young host larva, or during the interval from the second to the tenth day after oviposition. In some sectioned host larvæ the blastula stage of the parasites is found at the end of the sixth day, but this apparent precocious development occurs only in host larvæ which were about ready to hatch from the host egg at the time of oviposition by the parasite.

FIRST AND SECOND CLEAVAGE

The first cleavage of the segmentation or embryonic nucleus takes place at about the second day (Pl. 3, B). On the third day cleavage is found completed and two embryonic nuclei are visible in the embryonic region (Pl. 3, C). Each embryonic nucleus measures 3.8 μ in diameter, or about the same size as the original segmentation nucleus. The two paranuclear masses are larger; one of the masses is seen migrating from the anterior end to that part of the trophamnion which surrounds the embryonic region. The entire parasite body has increased slightly in size during the second day, and now measures about 19.2 μ in length and 9.1 μ in width.

The second cleavage of the embryonic nuclei takes place between the fourth and fifth days and produces four nuclei in the embryonic region (Pl. 3, D). Meanwhile the embryonic region has become spherical, and has increased in diameter to 10 μ. One of the paranuclear masses is now found beside the embryonic region.

During the fifth day the structure of the parasite body remains the same, but there is a notable increase in size of the parasite body, so that by the end of the fifth day the parasite body measures 22 μ in length and 11.4 μ in width (Pl. 3, F). The paranuclear masses are larger and usually widely separated in the trophamnion. At this time the parasite body is entirely surrounded by host tissue. This condition is true at least of most of the parasite bodies in a particular host (that is, say, four of six eggs originally deposited in the host), but others are to be seen in the same host which are not surrounded by host tissue, these being in a retarded and inactive stage of development. The retarded developing forms will be referred to later.

DIVISION WITHIN THE PARASITE BODY TO FORM TWIN GERMS

About the sixth day after oviposition the parasite body departs from its previous oval shape and becomes more elongate. The assumption of this newer shape is due to a division of each of the two paranuclear masses to form four separate masses. Two paranuclear masses are now to be found near or at each end of the parasite body.

A division of the embryonic region in some of the parasite bodies to form two separate embryonic regions then takes place, two of the four embryonic nuclei becoming included with each half of the divided embryonic region (Pl. 3, G). Each half of the embryonic region is now a true germ and is structurally the same as a 3-day-old parasite body after the first cleavage, since it is composed of two paranuclear masses and two embryonic nuclei in an embryonic region. No further division of the embryonic region or later developing blastula stages takes place; but each germ develops directly into the blastula and late embryo stages, and finally into a larva. The original egg (or parasite body) therefore develops into twin parasites.

SEPARATION AND DEVELOPMENT OF GERMS

Immediately after the division of the embryonic region to form twin germs, the trophamnion of the parasite body is still continuous (Pl. 3, H). Indentations of the trophamnion between the twin germs are soon visible, however (Pl. 3, G), and the two germs finally become structurally independent of each other. Henceforth each germ continues development by itself, although for a while both germs may continue to develop side by side until the blastula, or even a later stage, is reached, the two germs being held in contact or close together solely by the host tissue which surrounds them.

After the twin germinal regions are formed, the two embryonic nuclei in each germ (Pl. 3, G) divide and give rise to four embryonic nuclei (Pl. 3, H; Pl. 4, A). The embryonic region of each germ is spherical and measures about 12 μ in diameter when the four embryonic nuclei are just formed. The next cleavage of the embryonic nuclei results in the production of 8 nuclei in each germ. After the third cleavage, when 16 embryonic nuclei are to be found (Pl. 4, B), the nuclei become arranged in a circle (in section), thus assuming the typical early blastula stage. At this stage the embryonic region is still spherical and measures about 19.1 μ in diameter.

During the three cleavages of the embryonic nuclei the embryonic region of the germs is surrounded by the trophamnion. In the tro-

phamnion are two paranuclear masses that usually appear decidedly crescentic in shape (Pl. 4, A, B). The paranuclear masses continue to increase in size through their absorption of elements from the host tissue which surrounds the entire germ or early blastula.

THE ABORTION OF SOME EGGS

It has been shown above that on an average six to seven eggs are deposited by the female parasite in a host egg or newly hatched larva. If each of these developed twins successfully, the number of parasites produced would always be equal to twice the number of eggs deposited. Hence the average number of parasites reared from host puparia should be 12 or 14. It has been shown by the junior author (*1*), however, that, on the basis of the rearings of 100 puparia, the average number of cocoons produced in a single puparium is 6.52 and the average number of parasites reared is 6.31 per puparium.

One of the reasons why the number of parasites actually reared does not more nearly approximate 12 or 14 is the failure of some of the eggs to develop beyond the maturation and fertilization stages. It is common to find eggs in a stationary or degenerate stage of development at a time when other eggs in the same host, deposited at the same time,[6] are about to twin or have already passed the twinning stage. Figures E, F, and G of Plate 3 represent three types of eggs or parasite bodies found in a single host that originally contained five parasite eggs. Two of these five eggs have reached the twinning stage (one shown at G on Pl. 3); one egg has reached the stage in which it contains two paranuclear masses, and its embryonic region has not as yet divided (Pl. 3, F); and the fourth and fifth eggs have not developed to the stage of first cleavage of the segmentation nucleus (one shown at E on Pl. 3). Eggs of the latter type become aborted. They fail to develop, apparently because they did not become enveloped by host tissues from which they could receive the nutriment necessary to continue their development. At any rate, parasite bodies that are in the normal course of development at the cleavage or later stages are always observed surrounded by host tissue.

Aborted or degenerating eggs nearly always stain less deeply than normal eggs; their nuclei are smaller and frequently appear irregular around the edges, while the nuclei of normal eggs are regularly rounded. Approximately one-third of the eggs deposited by the parasite at one time become aborted.

MONEMBRYONIC DEVELOPMENT OF SOME EGGS

If twin germs were regularly produced from all nonaborted eggs, an even number of germs or blastulas should always be found in each host larva. That twinning is not the only process of development in this species is indicated by the finding of an odd number of germs or blastulas in perfect serial sections of parasitized larvæ. As examples, two sectioned hosts show seven germs each, a third shows seven blastulas, several show three blastulas, and others show either an odd or even number of both the germ and blastula stages of the parasite.

Moreover, a further study of the stage of the parasites in a host containing an odd number of parasites shows that the parasite bodies

[6] Oviposition into the host was observed under the binocular microscope and in most of the instances by far, only one oviposition was permitted in each egg.

within it are not all in the same stage of development. For example, one host larva containing three parasite bodies will show (1) that two of the parasite bodies are in the twin-germ stage, each embryonic region of which contains four embryonic nuclei (Pl. 3, H), and (2) that the third parasite body contains eight embryonic nuclei in an undivided embryonic region (Pl. 3, I). An older host larva containing seven germs or blastulas will show (1) four blastulas at the 16 or 32 cell stage, which by their paired association in the host and the fact that each pair is held together by host tissue are known to have developed by the twinning process, and (2) three isolated parasite bodies showing 8 or 16 nuclei in their embryonic regions, which by their shape (Pl. 4, C) and position in the host obviously developed from individual parasite eggs. Since twinning of a parasite body takes place only at the 4-cell stage of the embryonic region, and since an odd number of germs and blastulas is frequently found in the host, the conclusion can safely be drawn that some of the eggs of *Platygaster hiemalis* develop monembryonically.

The monembryonic process of development in *Platygaster hiemalis* is not unlike that described by Marchal (*4*) for the platygastrids *Synopeas rhanis*, *Trichasis remulus*, and *Platygaster ornatus*. In these species Marchal demonstrates how the embryo develops in a differentiated region of the egg, just as *P. hiemalis* does when it develops monembryonically. Moreover, the eggs of the above-mentioned species described by Marchal develop in a cyst of host tissue, nourishment from which is obtained by the trophamnion and the paranuclear masses and supplied to the growing embryo exactly as in *P. hiemalis*.

SUMMARY AND DISCUSSION OF CLEAVAGE TO BLASTULA STAGES

It has been shown above that after maturation and fertilization in the case of an inseminated egg an embryonic region becomes differentiated in the posterior part of the egg, and that after some growth of the parasite body, the embryonic region divides, two of the four embryonic nuclei passing to each of the two newly produced embryonic regions. Each of these two embryonic regions, together with its component paranuclear masses and trophamnion, develops into the blastula and finally into the larva stage, thus demonstrating a form of polyembryony in this species known as twinning. Moreover, certain of the eggs fail to develop beyond the segmentation nucleus stage and become aborted, probably because they do not become invested by host tissues from which they could absorb nourishing material necessary for their continued development. It has further been shown that some of the parasite bodies do not twin, but on the other hand develop monembryonically in a manner not unlike the monembryonic development described by Marchal for other platygastrid species.

Specialized monembryony.—It is obvious that *Platygaster hiemalis* exhibits both the highest type of monembryonic development and the simplest type of polyembryonic development yet known. In its monembryonic development, it is specialized to the extent that it must draw upon its host for nutriment during the course of development of the embryo, for the reason that its egg is too small (its yolk is therefore insufficient) to develop a larva to the point where it can feed for itself. Consequently the cortex of the differentiated embryonic region is utilized as a nutritive membrane (trophamnion), which, together with its paranuclear masses (of polar body origin), provides nutriment sufficient to

permit full development of the embryo at the expense of, but not to the detriment of, the host.

Twinning and specialized polyembryony.—In its twinning form of development, *Platygaster hiemalis* exhibits the simplest type of polyembryony, in that only two individuals are developed from a single egg or parasite body. Other polyembryonic Hymenoptera, such as *Copidosoma truncatellum* and *Copidosoma gelechiae*, develop 1,500 to 2,000 and 150 to 225 individuals, respectively, from a single egg. In these species the embryonic region does not divide at the end of the second cleavage of the segmentation nucleus, but instead the segmentation nucleus continues to divide within the original embryonic region, until it is a veritable syncytium composed of 200 or more embryonic nuclei. Then at a later stage, the trophamnion surrounding the embryonic region penetrates among the embryonic nuclei, and after portions of the trophamnion encompass one or two embryonic nuclei, the typical germs are formed. Disjunction of the many germs then takes place in a manner similar to that in the twin germs of *P. hiemalis*, but on a larger scale.

Platygaster hiemalis further differs from *Copidosoma gelechiae* and *C. truncatellum* in that the germ stage and later stages of *hiemalis* do not divide, while the morula stages of both *gelechiae* and *truncatellum* do divide. It has been shown by Leiby (*2*) that the morulas of *gelechiae* divide once, each daughter component finally developing into a parasite larva. The senior writer has also observed that the morula stage of *truncatellum* divides once and that the daughter morulas also divide, each tertiary morula giving rise to a parasite larva. Hence it is evident that the division of the morula stage in *gelechiae* and *truncatellum* is simply a specialization of the twinning process exhibited by *hiemalis*. This specialization by division results in the production, from a single egg, of a greater number of parasite individuals which the larger host is able to mature.

Aborted eggs and pseudo-forms.—As previously pointed out, of the six to eight eggs deposited in the host at the same time by *Platygaster hiemalis*, approximately one-third fail to develop beyond the segmentation nucleus stage, apparently because such eggs fail to become invested by host tissues. Such eggs degenerate. No stages beyond the egg ever degenerate, however, because the twinning and monembryonic processes of development are relatively simple. On the other hand, the complex and more specialized development of the parasite body of *Copidosoma gelechiae* and that of *C. truncatellum* results in the production of some degenerate germs, embryos, and larvæ [7] in these species, along with the normal forms that are destined to mature adult parasites.

It is probable that the development of other polyembryonic species will be described in the future, in which it will be shown that four, eight or more larvæ develop from a single egg. Such species will doubtless show that the number of degenerate or pseudo-forms of the embryonic stage developed will increase in proportion to the number of mature individuals originating from a single egg.

Monembryony and the origin of mixed broods.—The origin of single individuals of a sex different from that of all the other individuals reared in the same brood has been of some interest to those who have reared polyembryonic insects. Especially is this true of the species of

[7] These have been described by Leiby (*2*) in *Copidosoma gelechiae* as pseudogerms, pseudomorulas, pseudembryos, and pseudolarvæ. Similar forms have been observed by the senior writer in *C. truncatellum*.

Platygaster, which emerge in relatively small numbers from an individual host specimen. For example, on an average 6.31 adults of *P. hiemalis* issue from a single host carcass; and the mixed broods frequently show a sex ratio such as five females and one male, or seven females and three males. The rearings of 100 parasitized puparia show that 20 contained no males (pure female broods); and of the 80 mixed broods 23 contained one male, 25 contained two males, 12 contained three males, and 20 contained four or more males. In 71 of the 100 puparia the females exceeded the males in number, in 24 the males exceeded the females, while from the other five host puparia the males and females were reared in equal numbers. Unpublished studies on *Platygaster* sp. are available in notes of the senior writer which show a similar sex ratio.

Patterson (*6*, 7) has also pointed out the sex ratio of *Platygaster felti* Fouts, but in this species the average number of parasites (15 per brood) issuing from a single carcass is nearly three times as great as in *P. hiemalis*. *P. felti* oviposits one or two eggs in a single host egg and not from six to eight, as does *P. hiemalis*. The probabilities are that *felti* develops more than two parasites from a single egg, thus carrying the polyembryonic method of development beyond the twinning stage.

With reference to the development of mixed broods in *Platygaster felti*, Patterson has proposed the theory that both sexes arise from a single egg. He believes that the most probable way that this will be found to occur is by the Bridges method of somatic nondisjunction of the sex chromosome during cleavage of the embryonic nuclei.

The writers believe that the mixed broods of *Platygaster hiemalis* can be readily accounted for by the fact that both fertilized and unfertilized eggs are deposited in the host at the same time,[8] a condition which has been shown to occur regularly. The writers do not believe that this parasite controls insemination as the honeybee apparently does, but the short period of time required by the parasite to deposit a group of from six to eight eggs indicates the probability of the eggs passing the spermatheca duct so rapidly during oviposition that all of the eggs do not receive a sperm.

The writers believe also that the monembryonic development of some of the eggs of a group deposited by *Platygaster hiemalis* will account for the production of single males, or males in small numbers, in a mixed brood where females predominate, and similarly for single females or females in small numbers in a mixed brood where males predominate. In *P. hiemalis* the mixed broods more commonly yield a preponderance of females. To take a case in point, a common number of parasites reared in a mixed brood is 8, with a sex ratio of 6 females and 2 males. Studies of the number of eggs deposited by females in groups at the same time permit the writers to assume that in this instance 7 eggs were deposited in this group. Of the 7 it is also fair to assume that 4 were inseminated and 3 were not inseminated. If 2 of the 7 eggs became aborted in the course of development, and these 2 represented a fertilized and an unfertilized egg, there would remain 3 fertilized and 2 unfertilized eggs to develop successfully. The 6 females and 2 males of the mixed brood taken as an example would therefore have been produced by the twinning of each of the 3 fertilized eggs and the monembryonic develop-

[8] This fact can be readily ascertained with accuracy by examining the eggs of a group deposited by the parasite. All the eggs of such a group are identically fixed and stained, and their smallness of size permits several eggs of the group to be contained side by side within a single section cut 6μ thick. Under these conditions some of the eggs of a group show a sperm within while others show no sperm.

ment of the 2 unfertilized eggs; assuming, of course, that the fertilized eggs produced females and the unfertilized eggs produced males. If there had been 3 males instead of 2 to account for from the 2 unfertilized eggs, 1 of the eggs might have developed twins while the other developed a single male by the process of monembryony.

Admittedly, the brood taken above as an example is only one of many ratios of the sexes in which the adult parasites are bred from host puparia. But just as the number and the sex of the individuals reared from different host puparia are found to vary, so will the factors underlying the development of the individuals of a brood vary. These factors are (1) the number of eggs in the group deposited in the host, (2) the number of eggs inseminated, (3) the percentage of the eggs becoming aborted, and (4) whether the eggs that develop will follow the twinning process or will develop monembryonically. A study of many combinations of these factors, as found in various sectioned host eggs and young larvæ,[9] leads to the conclusions (1) that mixed broods originate from fertilized and unfertilized eggs deposited in the host at the same time by a fertilized parasite, and (2) that the monembryonic development of some of the eggs accounts for the rearing of one, two, three, or four males or females in a mixed brood in which the number of the individuals of the opposite sex predominates.

BLASTULA TO LARVA STAGES

To follow the detailed development of the parasites from the blastula to the larva stages is in itself an extensive problem, and only a very general account of this development can be given here.

The parasites continue their development from the blastula stage during the fall months (Pl. 4, D, E), and early winter finds them in the advanced embryo stage in which they exhibit larval characteristics (Pl. 4, F; Pl. 5, A, B). During this interval all of the embryos which have arisen by the twinning process become separated structurally from each other, although a twin pair may still be located side by side (Pl. 5, A). Each embryo is found in an embryonic cavity the outer lining of which is the trophamnion. During the development of the embryo the trophamnion is relatively thick and contains many small (Pl. 4, E) or two large conspicuous paranuclear masses. The parasites pass the winter as well-formed embryos distributed between the fat bodies of the host, which has meanwhile become fully grown and encased in a puparium on the wheat plant.

In spring the parasites continue their development. The embryos straighten out from their previous U-shaped form and are recognized as young larvæ. While this development is taking place, the trophamnion becomes relatively thinner and its paranuclear masses are absorbed until it is represented merely by a very thin membrane surrounding the young larva (Pl. 4, G).

In the latter part of spring, the young larvæ rupture the trophamniotic membrane and begin to feed upon the body content of the host. By the time the entire content of the host is devoured, the larvæ are full-grown. The larvæ remain within the body wall of the host during early summer, when each larva forms a chamber or cell in which it transforms to a pupa. The formation of the pupal chambers, which correspond in size to that of the larvæ, distends the body wall of the host abnormally, the

[9] The preparations originated from host eggs which had been oviposited in by parasites under laboratory controlled conditions. The parasites were reared to the advanced blastula stage in the laboratory.

host integument becoming so thin that the outlines of the parasite chambers can readily be discerned (Pl. 1, D).

The parasites remain in their chambers in the pupa or adult stage throughout the remainder of summer. They emerge as adult parasites from the host carcass and puparium in early fall, to deposit their eggs in eggs of the Hessian fly, a brood of which emerges at the same time.

SUMMARY

(1) *Platygaster hiemalis* develops both monembryonically and polyembryonically in the Hessian fly, the adult parasites emerging in late summer from the host puparium. An average of 6.31 individuals, often of both sexes, is bred from each puparium.

(2) The female parasite deposits a group of from four to eight eggs at one oviposition in the egg and occasionally in the young larva of the host during the fall of the year. Some of the eggs of the same group are inseminated while others are not inseminated.

(3) During maturation two polar bodies are formed in the egg. These unite to form a single polar nucleus in the anterior region of the egg. Maturation is identical in fertilized and unfertilized eggs.

(4) After maturation the female pronucleus fuses with the male pronucleus to form a cleavage nucleus, which becomes located in the posterior part of the egg. The female pronucleus of an unfertilized egg is similarly found in the posterior region.

(5) Part of the egg containing the cleavage nucleus then becomes differentiated from the remainder of the egg. This differentiated part is the embryonic region, which together with its contained cleavage or embryonic nucleus gives rise to one or two embryos. The remainder of the egg, containing the polar nucleus, is homologous to the trophamnion and paranucleus of previously described polyembryonic insects. Its function is to nourish the embryos until they are young larvæ and can feed for themselves upon the host.

(6) The embryonic nucleus divides to form two and then four embryonic nuclei. At the same time the polar nucleus divides to form two polar nuclei or paranuclear masses and these divide to form four such masses.

(7) The embryonic region of some of the eggs or parasite bodies then divides to form two embryonic regions, and one region together with two of the four paranuclear masses becomes separated from the other, although both may continue development side by side for some time, being held together by host tissues. This division of the parasite body results in the formation of twin germs, each of which develops directly into a blastula, then into a late embryo stage, and finally into a parasite larva.

(8) The embryonic region of other eggs does not divide. Such eggs develop a single parasite by the monembryonic process which is similar to that described for other platygastrids.

(9) Approximately one-third of the eggs deposited do not develop beyond the cleavage nucleus stage, probably because they fail to become invested by host tissue.

(10) The twinning development in insects here described for the first time is a simple type of polyembryony. On the other hand, the monembryonic development of this parasite is very highly specialized. Since

Platygaster hiemalis exhibits both types of development, it furnishes a clue to the origin of polyembryony in insects.

(11) It is believed that the monembryonic development of some of the eggs, and the fact that some of the eggs of a group are inseminated while others of the same group are not, will account for the origin of mixed broods of the parasites, and the occurrence of single individuals of a sex different from that of the others of the brood.

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PLATE 1

Platygaster hiemalis

A.—Outline of egg of Hessian fly showing two groups of Platygaster eggs deposited within. $\times$ 460.

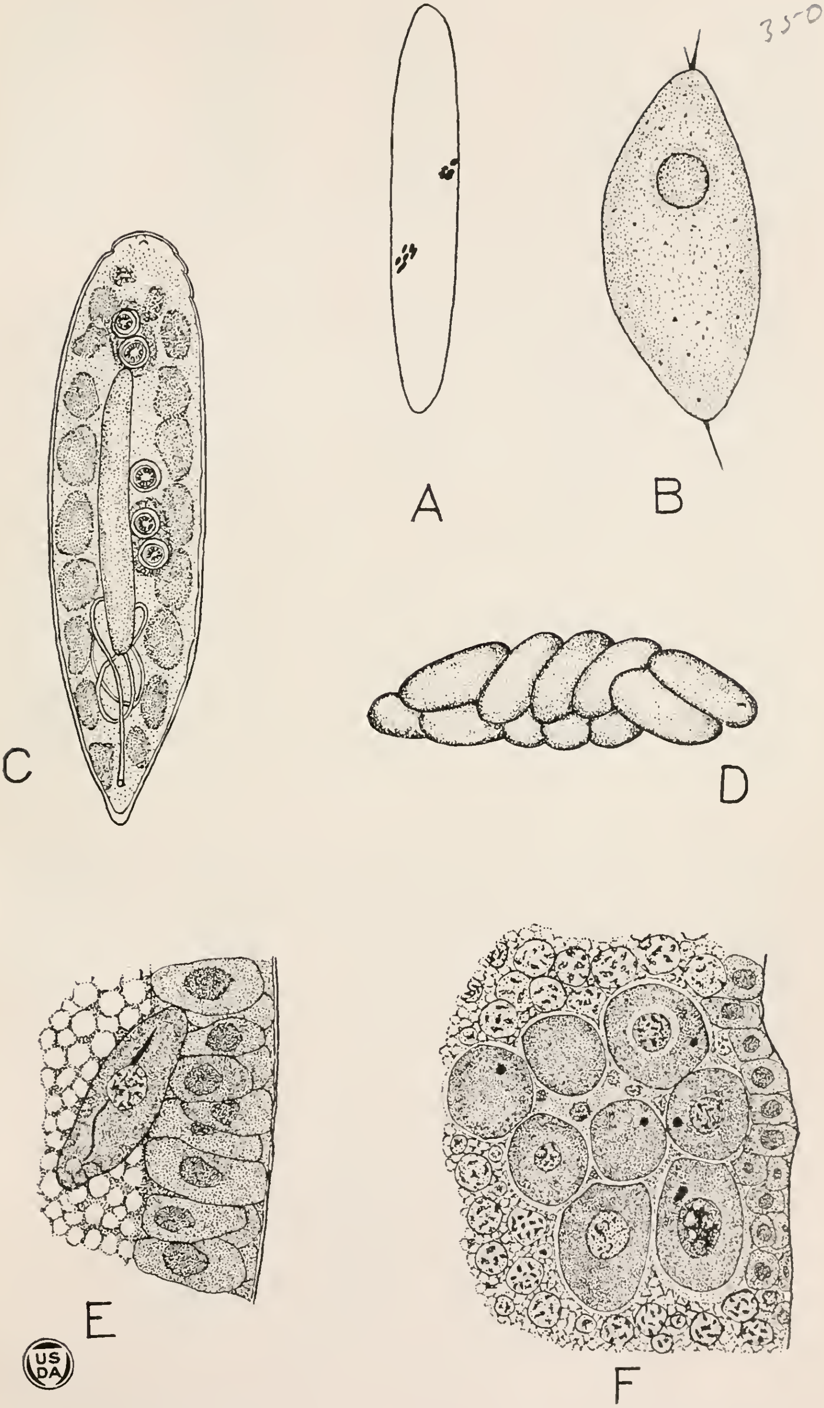
B.—Preoviposited egg of *Platygaster hiemalis*. Note the membranous process at each end and the egg nucleus. $\times$ 2,300.

C.—View within a larva of the Hessian fly, showing five Platygaster parasites in the blastula stage. $\times$ 35.

D.—Host carcass containing 11 cocoons of Platygaster. $\times$ 13.

E.—Parasite egg immediately after oviposition, with nucleus and sperm. Egg was deposited in the yolk and under the blastoderm of the host egg. Compare size of egg with that of blastoderm cells. $\times$ 2,200.

F.—Portion of a section through a host egg cut across a group of eight parasite eggs which were deposited at one time. Five of the eight eggs show sections of the sperm. $\times$ 2,200.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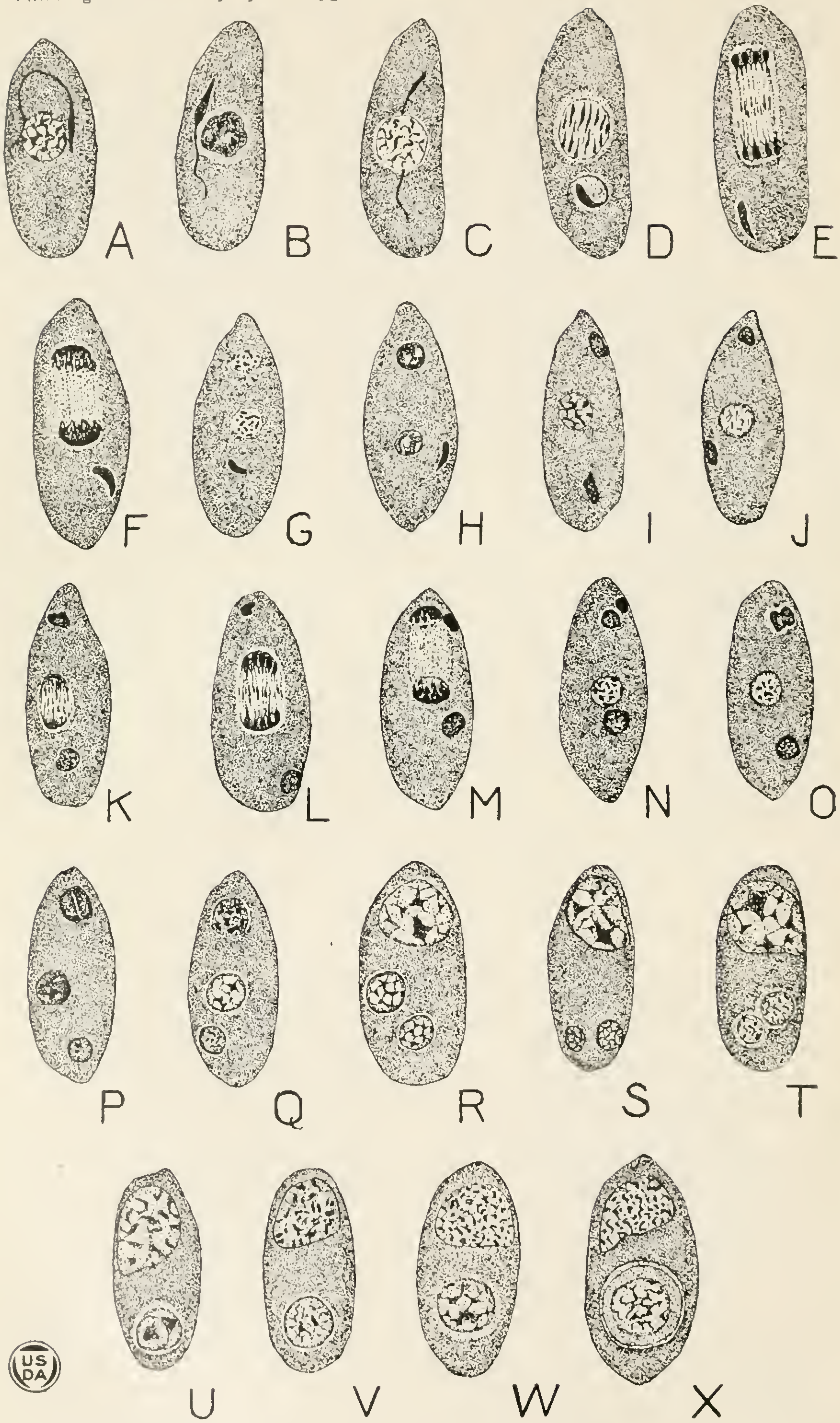
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PLATE 2

Platygaster hiemalis

All figures drawn 2,255 times natural size.

A.—Egg one hour after deposition, with nucleus and sperm.

B.—Egg about one and one-half hours after deposition. Nucleus condensing.

C.—Egg 2 hours old. Chromosomes of nucleus are again evident.

D.—Egg 4 hours old. Nucleus is at prophase and sperm is curled.

E.—Egg about 4 hours old, showing first maturation spindle. The sperm or male nucleus is spindle-shaped.

F.—Like figure E but at a slightly later stage. The anterior part of the spindle becomes the first polar body and the posterior part becomes the oocyte nucleus of the second order.

G.—Egg showing first polar body which remains in the egg, oocyte nucleus, and male nucleus.

H.—Like Figure G but at slightly later stage.

I.—Egg about six hours after deposition. Polar body condenses to become an irregularly shaped mass of chromatin while the oocyte nucleus of the second order expands slightly.

J.—Like Figure I, but at slightly later stage.

K.—Egg about 7 hours old, showing the first polar body, second maturation spindle, and male nucleus.

L.—Seven-hour-old egg.

M.—Second maturation about complete. The anterior part of the spindle becomes the second polar body while the posterior part becomes the female pronucleus.

N.—Egg eight hours after deposition. The first and second polar bodies are found close to each other in the anterior end of the egg, which is known as the polar region.

O.—Egg 10 hours old. The first and second polar bodies approach each other. Female pronucleus is in center of the egg and male nucleus is usually in the posterior or embryonic region of the egg.

P.—Slightly later stage than Figure O. Polar bodies are fusing.

Q.—Egg about 11 hours after deposition, with a single polar nucleus (two fused polar bodies) in anterior end. The male and female pronuclei expand.

R.—Egg 12 hours old. Polar nucleus elaborates in anterior end of the egg.

S.—Like Figure R, but male and female pronuclei are found close to each other in the posterior region of the egg. The male pronucleus is always smaller than the female pronucleus.

T.—The male and female pronuclei are about to fuse.

U.—Fusion of pronuclei in posterior region of egg. The chromatin of the polar nucleus breaks up.

V. Parthenogenetic egg 24 hours old showing polar nucleus, and segmentation nucleus which has maturated twice like fertilized egg.

W.—Fertilized egg about 24 hours old, with polar nucleus showing particles of chromatin scattered over a reticulum and segmentation nucleus in a resting stage. The egg is usually surrounded by host tissue at this stage.

X.—Egg from 1 to 2 days old. During this interval the embryonic region containing the segmentation nucleus becomes differentiated in the posterior part of the egg.

PLATE 3

Platygaster hiemalis

A. Like X on Plate 2, but the egg has increased in size and is henceforth known as the parasite body. During the one to two day interval the polar nucleus divides to form two paranuclear masses. The polar region and the part of the parasite body surrounding the embryonic region is known as the trophamnion. ×2,200.

B.—Section of a parasite body between two and three days after oviposition. The segmentation nucleus divides to form two embryonic nuclei. ×2,200.

C.—Parasite body about 3 days old, showing two embryonic nuclei in the embryonic region. One of the paranuclear masses begins to migrate in the trophamnion toward the embryonic region. ×2,200.

D.—Parasite body four or five days after oviposition. The two embryonic nuclei of the previous stage have divided to form four embryonic nuclei. ×2,200.

E.—Section of an aborted and degenerating egg. ×2,200.

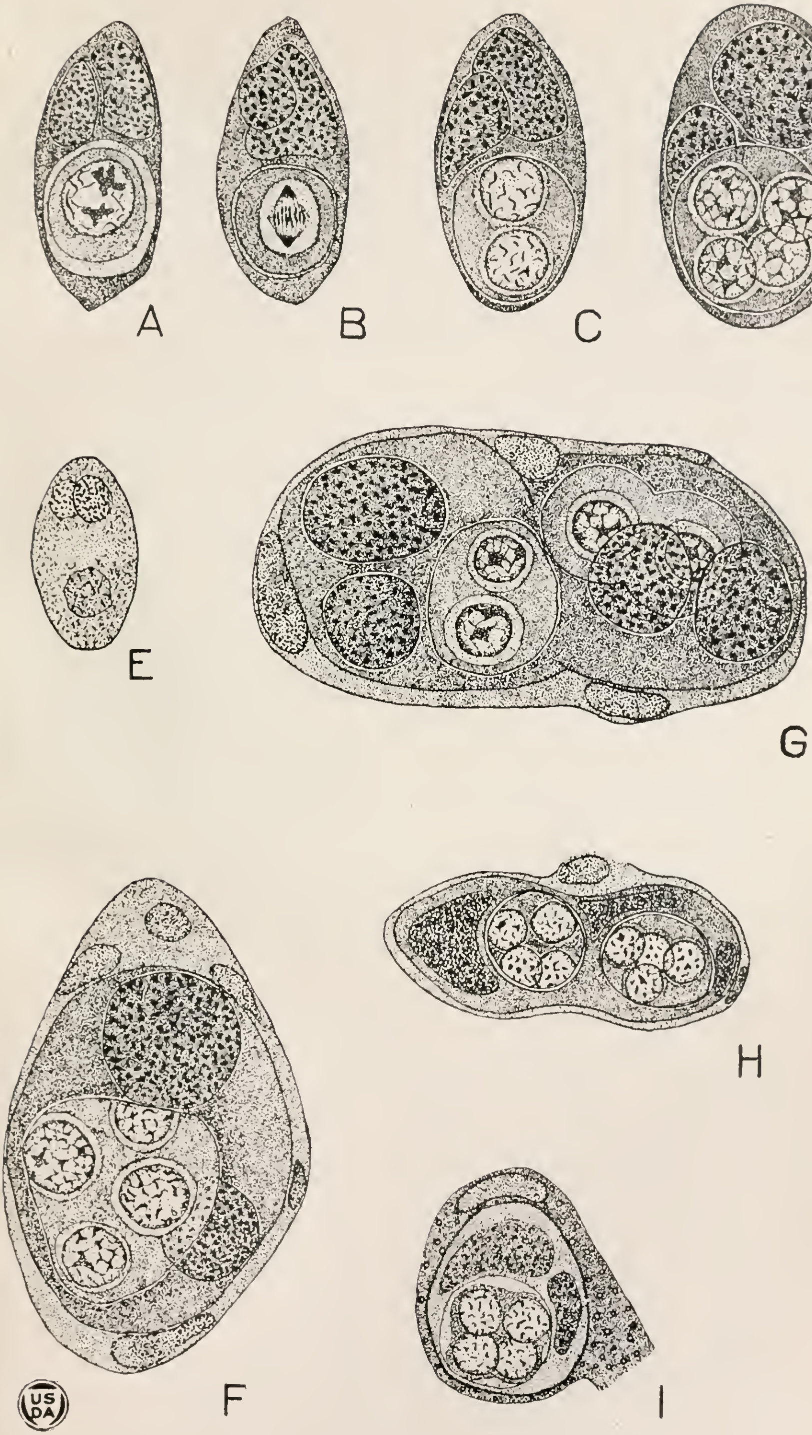
F.—Composite drawing of two sections of a 6-day-old parasite body showing four embryonic nuclei in the embryonic region. One of the two paranuclear masses is shown lying in the trophamnion beneath the embryonic region. The parasite body is entirely surrounded by a cyst of host tissue.

G.—Section through the twinning stage of the parasite body, which takes place about six days after oviposition. The embryonic region has divided, two of the four nuclei passing to each half of the twin embryonic regions. The division of the two paranuclear masses forms four similar masses. Two paranuclear masses and one of the embryonic regions with its two nuclei thus form a germ, and the twin germs become separated structurally after the trophamnion infiltrates between the two embryonic regions. ×2,200.

H.—A twin germ surrounded by host tissue with the two embryonic regions still encased in a common trophamnion. ×1,100 (half the magnification of the preceding figures).

I.—Parasite body which is developing monembryonically while encased in a part of the host salivary gland. Four of its eight embryonic nuclei are shown in this section. ×1,100.

PLATE 3



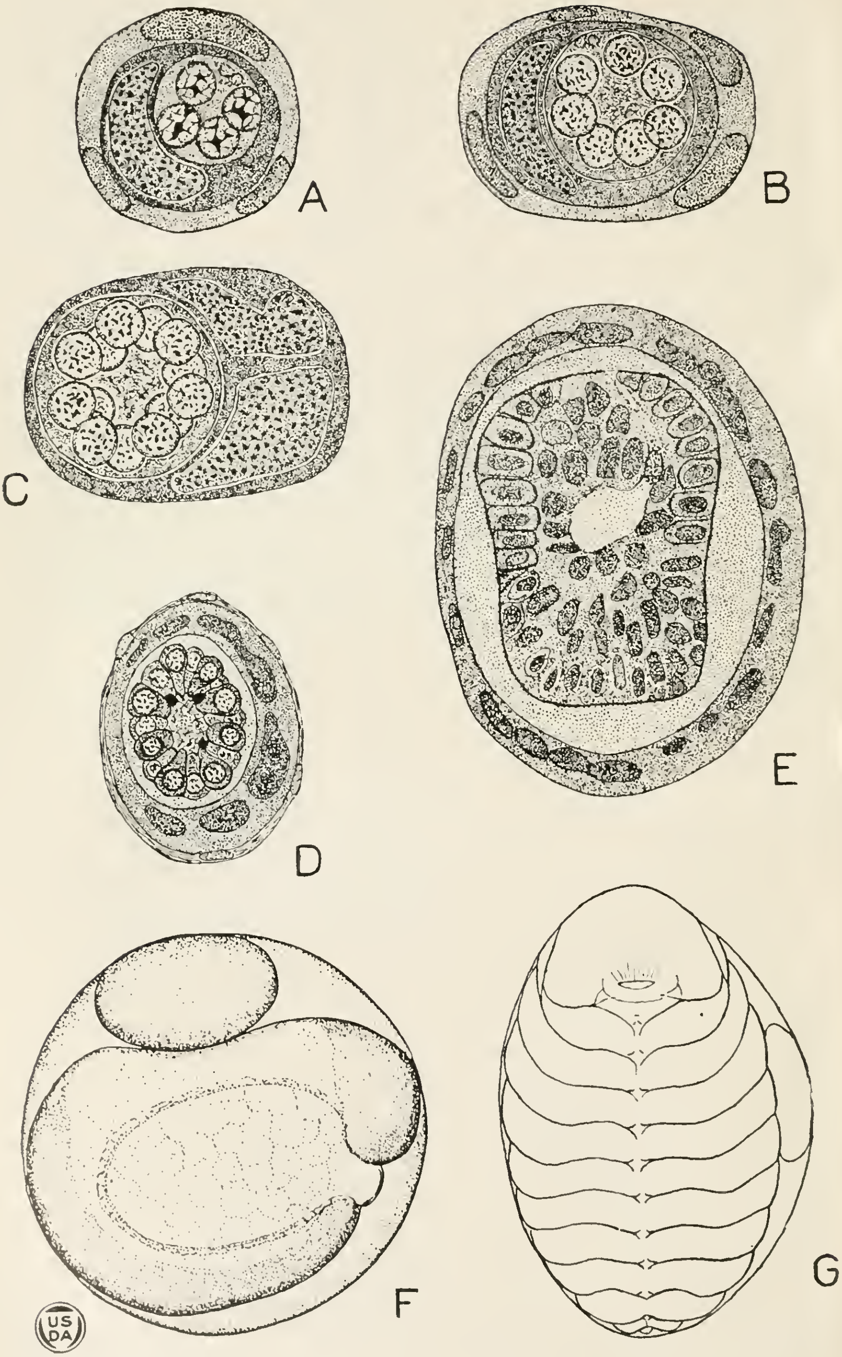

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PLATE 4

Platygaster hiemalis

A.—One germ of a twin that has become disjoined from the other. It is surrounded by host tissue and its embryonic region contains four nuclei. One paranuclear mass is shown in the trophamnion. ×1,100.

B.—Embryo in the early blastula stage containing 16 embryonic nuclei, of which 7 are shown. ×1,100.

C.—Monembryonically developing egg in 32-cell blastula stage with two paranuclear masses in the trophamnion. Host tissue omitted. ×1,100.

D.—Blastula stage of embryo. Note the breaking up of the paranucleus into smaller masses. ×550.

E.—Embryo at the time of formation of germ layers. ×550.

F.—En toto drawing of U-shaped embryo within its trophamnion. One large paranuclear mass is located above it. ×108.

G.—Outline drawing of a parasite larva with paranucleus. The trophamnion has become reduced to a thin membrane. ×60.

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PLATE 5

Platygaster hiemalis

A.—Photomicrograph of twin embryos which have developed side by side between fat bodies of the host. The embryos are typically U-shaped and slightly advanced in development of the one shown at F on Plate 4. × 140.

B.—Photomicrograph of a section through three embryos in one end of a host larva. Note the thickened trophamnion of the embryo on the left. × 116.


A
B

PATHOGENICITY OF OPHIOBOLUS CARICETI IN ITS RELATIONSHIP TO WEAKENED PLANTS[1]

By H. R. Rosen and J. A. Elliott, *Arkansas Agricultural Experiment Station*

In the United States the "Australian take-all" of wheat was first reported in 1920[2] (*6*),[3] from New York. The following year it was discovered by the writers in Arkansas.[4] As the field observations strongly suggested that the fungus, *Ophiobolus cariceti*, found on diseased wheat, was a weak parasite, attacking plants which were more or less unthrifty, experiments were undertaken to obtain more information on this point.

When Ophiobolus was first found in this country it was regarded with such alarm that gasoline was poured over the infested area and the plants burnt (*6*). This measure doubtless was justified inasmuch as the disease had been considered a serious one by investigators in Australia and in European countries, and the discoverers may have had in mind the possibility that the disease had been recently introduced into this country and was confined to a small area.

In May, 1921, Mr. O. Pool, a farmer living about 4 miles west of Fayetteville, Ark., brought into the laboratory diseased wheat showing marked blackening at the bases of the culms. (See Pl. 1 and 2.) The plants were much stunted, and the roots were largely dead and bunchy, with abnormal development of woolly root hairs on parts adjoining the stools. Blackish crusts of mycelium surrounded bases of culms, and extended into the enveloping leaf sheaths, and, finally, black, beaked, fruiting bodies, quite noticeable under a hand lens, were found submerged in the lower parts of the sheaths; when these were examined under the microscope, asci and spores, typical of Ophiobolus, were found.

FIELD OBSERVATIONS

The writers immediately went to the field from which the plants had been obtained and saw 11 acres of very sickly looking wheat. The field was undulating, with elevated spots on the eastern and western borders and a low area in between, which, according to Mr. Pool, became a good sized pond during rainy seasons, being flooded with water for two or three months at a time. The soil even on the elevations appeared poorly drained, with crawfish holes very noticeable. It is classed as Gasconade silt loam, a type, grayish white in appearance, which is usually poorly drained in this region, often giving an acid reaction, as this soil did.

The stand of wheat as a whole was poor (Pl. 3), the heads, even on the best plants, were undersized (by the end of May, wheat is usually well developed in this latitude), and bare spots were noticeable throughout the field. Many of the plants were stunted, 6 and 12 inch plants were bearing heads, and were either dead or about to die;

[1] Accepted for publication June 25, 1923.
[2] The disease of wheat first found in Madison County, Ill., in 1919 and at one time considered as "Australian take-all," or "so-called take-all," is now considered by McKinney, Eckerson, and Webb (*8*) as a "rosette disease" associated with intracellular bodies comparable to those found in various mosaic diseases. Stevens (*11*), in considering the footrot stage of the disease, has concluded that this is due to Helminthosporium.
[3] Reference is made by number (italic) to "Literature cited," p. 358.
[4] U. S. Department of Agriculture. Bureau of Plant Industry. Plant Disease Survey. wheat. the take-all survey. *In* U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Bul., v. 5, p. 4. 1921. (Mimeographed.)

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the leaves were yellowish, straw colored, markedly withered, and harbored any number of different fungi. Cladosporium and Alternaria spp. were so common on the leaves and heads as to give the plants a distinct, mouldy appearance. A careful survey of the field made on this trip and on subsequent visits showed Ophiobolus present at the base of many plants growing on the elevations. Numerous plants growing in the lower parts of the field appeared just as sickly as those on the knolls, but in contrast showed no evidence of the presence of Ophiobolus. There was but slight dark discoloration at the crown, and no black fungus crusts or fruiting bodies were found. The variety of wheat grown was Marvelous. It is a soft, winter wheat, not infrequently used in this section. The seed had been procured from a local grower who had obtained good yields from the same strain in previous years. This field had not grown wheat previously for 20 years and had been used as a pasture of red top and timothy (largely red top) for the past 12 or 14 years.

Mr. Pool had also another field of wheat, about 500 yards south of this, which had been grown from the same lot of seed, but this field, in contrast to the one just described, showed normal, healthy looking plants; it gave one of the best yields in the county. The soil here was of a different type, darker in color, well drained, and it had received an application of manure the previous year. It seems proper to conclude that as far as these two fields are concerned the seed played no part in the introduction and development of the disease. Furthermore, it is evident that as far as ecological factors are concerned the only known difference was that of soil condition.

Shortly after Ophiobolus was found on this farm it was discovered by the senior writer on another, near Prairie Grove, about 10 miles away. Here also the field was undulating and about 11 acres in size, but, unlike the first, Ophiobolus was confined entirely to certain spots at the north end. This portion represented the highest part of the field, the soil of which in color, texture, moisture content, and acidity was comparable to the Pool field. Ophiobolus was sharply confined to spots in a 3-acre area which had previously been used as a peach orchard. A fence row had formerly delimited the southern edge of this area and had been removed shortly before the land had been prepared for wheat the previous fall. The whole 11-acre field had then received the same treatment, and the drill rows had been run north and south, extending across the area that had previously been in peach trees and continuing down through the remainder of the field. The remaining 8 acres had been used for wheat in 1920, corn in 1919, and clover for 3 years previous to that. As compared with the north end of the field, the stand of wheat on this portion was thicker, the plants were larger, and the heads better filled. The soil was considerably better physically; it appeared well drained, there were no crawfish holes, and the subsoil instead of being a stiff clay [5] of a grayish color was more or less friable, reddish brown, and not watersoaked.

As just stated, Ophiobolus was found only in the 3-acre portion at the north end. Here the wheat as a whole was much poorer, and the general appearance of the plants closely resembled that on Mr. Pool's farm. Here also many stunted and badly diseased plants showed no signs of Ophiobolus. One of the worst infested spots in the field was a strip of

[5] The writers wish to express their thanks to Mr. R. H. Austin of the agronomy department, University of Arkansas, for taking samples of the soil and subsoil.

land running along its entire width through which the fence row had previously passed; part of the fence still existed to the west of the wheat field. This row marked the southern edge of an incline, and to the south of it the area was quite level throughout, and of a different type of soil, as above stated.

While Ophiobolus, both perithecia and mycelium, could be found scattered in the north end, a very careful search beyond the edge of the incline to the south failed to reveal a single plant with symptoms of Ophiobolus. The field was revisited several times; on one occasion the writers were accompanied by two other plant pathologists, Dr. A. G. Johnson and Mr. H. H. McKinney, and no evidence of attack by Ophiobolus could be found by anyone south of the region where the fence row had existed. As will be fully described later, a number of wild grasses were also found badly attacked by Ophiobolus in this same strip. In spite of the excellent opportunity for infection taking place on both sides of this strip, the drill rows extending through from the poor soil to the good soil, infections were sharply confined to the wheat growing to the north upon the poor soil. Here, as on the Pool farm, there appeared to be a close correlation between the presence of weak, sickly plants and the pathogenicity of Ophiobolus.

OPHIOBOLUS CARICETI ON WILD GRASSES

In addition to wheat, the following grasses have been found attacked: *Festuca octoflora*, *Festuca elatior*, *Bromus secalinus*, *Hordeum pusillum*, and *Chaetochloa geniculata*. Dr. A. G. Johnson first detected signs of Ophiobolus on a wild grass in the diseased wheat fields near Prairie Grove, previously mentioned, and while no fruiting bodies were observed at the time, a careful inspection by the senior writer of material subsequently gathered on the same field revealed typical fruiting bodies on two wild hosts, *Festuca octoflora* and *Bromus secalinus*. (Pl. 4.)

In the same strip of soil where the fence had previously existed, as well as in other spots in the north end of the field, many plants of *Festuca octoflora*, *Bromus secalinus*, and *Hordeum pusillum* were found showing symptoms similar to the diseased wheat in the same vicinity. A thorough search for Ophiobolus on wild grasses near take-all diseased wheat on the Pool farm also brought to light many diseased plants of *Festuca octoflora* (a common grass in this locality), on some of which perithecia of Ophiobolus were found.

As the presence of Ophiobolus on these wild grasses strongly suggested an organism more or less endemic in nature, a search was made for it in regions where wheat had not been grown. It was shortly after detected on unthrifty plants of perennial foxtail (*Chaetochloa geniculata*), growing in a few water-logged areas on the campus of the University of Arkansas. No further search was made, but as no wheat had ever been grown on the campus (used as such for about 50 years) there is no reason for doubting the endemic nature of Ophiobolus on this native grass.

IDENTITY OF THE ASSOCIATED ORGANISM

The question as to whether or not the species of Ophiobolus which attacks wheat is the same as that on the wild grasses was determined by a careful comparison of perithecia, asci, spores, and mycelium found on the different hosts. An illustration of the close morphological agreement may

be seen by the following measurements of asci and ascospores found upon three different hosts.

	Asci (μ).	Ascospores (μ).
Wheat	75.0 to 105.0×10.5 to 13.5	60.0 to 90.0×3.0 to 3.5
Chaetochloa	80.0 to 100.0×10.2 to 13.0	61.0 to 87.0×3.0 to 3.5
Festuca	85.0 to 100.0×10.0 to 13.0	61.0 to 88.0×3.0 to 3.5

These measurements are also in close agreement with those given by Fitzpatrick, Thomas, and Kirby (*4*). As these authors have not considered the species of Ophiobolus previously described which is found on wild grasses in this country, it seems desirable to present briefly the data obtained by the senior author on this phase of the subject.[6] Of the species of Ophiobolus that have been described as occurring on wild grasses the following deserve consideration: *O. Andropogonis* E. & E., and *O. Festucae* Tracy and Earle. Through the kindness of Dr. F. J. Seaver of the New York Botanical Garden type material of *O. Andropogonis* was carefully examined and compared with Arkansas material. As far as measurements of asci and ascospores are concerned, Ellis and Everhart's (*3*) species might readily be taken for underdeveloped material of *O. cariceti*, but the perithecia with large beakless osteoles, the absence of any blackish mycelium on the host tissue in which the perithecia are buried, and the apparent saprophytic nature of the fungus, are sufficient to mark it as distinct. Examination of co-type material of *O. Festucae* Tracy and Earle (*13*) found in the Missouri Botanical Garden shows two different fungi present, but the one, doubtless, which was described possesses asci and ascospores very much like those to be found in Ophiobolus species. However, the perithecia instead of being beaked or papillate, open by means of a cleft in the host epidermis. The fungus belongs to the genus Lophodermium and perhaps is close to *L. arundinaceum* (Schrod.) Chev. var. *alpinum* Rehm. Other species of Ophiobolus which have been considered, but which, judging from the descriptions, are very different from *O. cariceti* are: *O. eucryptus* (B. & Br.) Sacc., *O. leptospermus* (*Speg.*) Sacc., *O. trichisporus* E. & E., *O. medusae* E. & E., *O. paludosus* (*Feltg.*) Sacc. & D. Sacc., *O. herpotrichus* (Fr.) Sacc., *O. coffeatus* (*Berk.*) Sacc., *O. stictisporus* (Cook & Ellis) Sacc., *O. littoralis* (Crouan) Sacc., *O. culmorum* (Crouan) Sacc., and *O. helicosporus* (B. & Br.) Sacc.

PATHOGENICITY OF OPHIOBOLUS

As all the field observations suggested that Ophiobolus attacked weak, poorly growing plants, it seemed worth while to test the effect of different fertilizers in a field where Ophiobolus was present. For this purpose 4 acres of the field in which Ophiobolus existed were set aside by Mr. Pool for our use. The 4 acres chosen consisted of two areas of similar size, one in the southeast corner and the other in the northwest corner of the field. These parts were selected because wheat attacked by Ophiobolus had been found to be most abundant on them and the fungus appeared equally distributed throughout. The chosen areas were divided into 1-acre plots, each of which received the following: Plot 1, no treatment; plot 2, 10 tons of manure; plot 3, 1 ton of burnt lime; plot 4, 400 pounds of commercial fertilizer of a 4–8–3 formula. The manure, lime, and commercial fertilizer, with the exception of sodium nitrate, which was applied the following March, were disked in at the same time, in the fall, and all the plots otherwise received the same treatment throughout.

[6] We are indebted to Dr. E. A. Burt for the use of the splendid library and mycological herbarium of the Missouri Botanical Garden.

In order to ascertain any difference in varietal susceptibility in wheat, as well as the pathogenicity of Ophiobolus on *Festuca elatior*, each plot was divided into four equal parts, sown to grass and wheat. One of these parts in each acre plot was sown to Festuca, another to Pool's Marvelous (wheat which had been grown on this field the year previous), another to Alabama Blue Stem, and the remaining equal part of each plot to Marvelous.

The plots were visited[7] frequently and notes kept of their appearance at different times. The first marked differences were noted on December 10, 1921. Among the notes taken on that day are the following: Manure plot, plants 4 to 5 inches tall and of good color; stand is good, Alabama Blue Stem showing up the best. Lime plot, much like the untreated plot; stand is poor, plants are off color and lacking in vigor. Commercial fertilizer plot, stand is excellent; the plants are of good color and vigorous. Untreated plot, stand poor; plants are undersized, spindling, and off color.

From December on through the winter and spring the same differences prevailed. On April 18, when the plots were photographed (see Pl. 5, A and B), those treated with manure and commercial fertilizer showed excellent growth as compared with the untreated and limed plots. A few of the plants in the manure as well as in the commercial fertilizer plots appeared undersized, but it was impossible to tell whether this was due to improper distribution of the manure and fertilizer in the vicinity of such plants, or to some other cause. On June 8, when the heads were almost mature, it was possible to make actual counts of the number of plants which clearly possessed symptoms of Ophiobolus. The amount of infection was as follows: Untreated, 80 per cent; manure, 45 per cent; commercial fertilizer, 7 per cent; lime, 95 per cent. When the wheat was harvested and threshed the following yields were obtained:

Variety.	Yield from untreated plot.	Yield from plot treated with manure.	Yield from plot treated with commercial fertilizer.
	Bushels.	*Bushels.*	*Bushels.*
Pool's Marvelous	0.8	3.0	5.5
Alabama Blue Stem	1.2	5.3	5.0
Marvelous	1.1	2.0	3.3

The lime plot was a total failure; the plants were so stunted and the stand so poor that it was considered useless to attempt to harvest the crop. The figures shown above represent actual yields of each one-fourth-acre plot. The one-fourth acre of Festuca which was included in each plot is not figured, since the stand on all was poor, weeds having taken possession of the soil before the plants had made any growth. Figuring on the basis of yield per acre and averaging the three varieties, the untreated plot yielded 4 bushels, manure plot 14.3 bushels, and commercial fertilizer 18.4 bushels (Pl. 5). The yield from the manure plot is above the average for the State, while that from the commercial fertilizer was considered so phenomenal by the farmers in the vicinity that it attracted

[7] The writers are greatly indebted to Mr. R. F. Crawford, who spent much of his time in seeing that these experiments were properly carried out, and in keeping careful notes on the various plots.

a great deal of attention. The yield of the control plot was comparable to that obtained by Mr. Pool on the same area the previous year.

DISCUSSION

The writers have no satisfactory explanation to offer for the presence of the Ophiobolus on some of the plants of the manure as well as the commercial fertilizer plots, but whatever such an explanation may be, it is clear that irrespective of the presence of the fungus the yields on these plots as compared with those on the untreated and lime plots were so satisfactory that for practical purposes these treatments, particularly the commercial fertilizer, may be considered as having almost completely controlled the disease. The percentage of infection on the manure and commercial fertilizer plots as compared with that on the check and lime plots was so strikingly different that there can be no question that the parasitism of Ophiobolus was greatly inhibited. Why commercial fertilizer gave better control than manure can not be answered at this time, but it is perhaps safe to conclude that control may be not entirely a matter of a sufficient amount of nutrients.

The pathogenicity of *Ophiobolus cariceti* (*O. graminis*) on healthy plants has been questioned by various investigators previous to this time. From Stevens' (*10*) excellent summary of the literature on wheat footrots, including Australian take-all, the following references may be noted: Pearson (*9*) in 1888 decided that this is a poverty disease, "and that the fungus which causes take-all attacks mainly such crops as are insufficiently nourished." Tepper (*12*) in 1892 said that "Take-all is nothing else than starvation of the crop." McAlpine (*7*) in 1902 stated that "Take-all largely depends on the nature of the season and the mechanical condition of the soil. * * * If the soil is neither too dry * * * nor so wet as to cake * * * and contains sufficient plant food * * * then the take-all will not appear." Voges (*14–18*) in a number of articles running over a period of years up to 1914 concludes that Ophiobolus is not the primary cause of the disease, but, judging from his descriptions, it may be doubted whether he had studied the Ophiobolus disease which is described by McAlpine and others as common in Australia. Various other investigators who have associated Ophiobolus with this disease have concluded that conditions which tended to weaken the plants, such as frost, wet weather, poor soil, etc., rendered the plants susceptible to attack by this fungus. Most of them, however, rely almost entirely on field observations, and little or no attempt has been made to study these relationships under rigid experimental conditions with adequate controls.

Contrary to the opinion that Ophiobolus attacks only weakened plants, the recent paper by Kirby (*5*) presents the view that the fungus is a vigorous parasite capable of attacking healthy plants, and cites the work of other investigators who express or imply similar views. Inasmuch as Kirby's work is much more thorough and exact than that of any of the others it may suffice to analyze his work. Using pure cultures of *Ophiobolus cariceti* growing on wheat kernels, he inoculated 156 pots of wheat at planting time and noted that at maturity the plants in all the inoculated pots showed typical symptoms of take-all, while no plant in any of the 78 control pots exhibited such symptoms. From the inoculated plants he then recovered the same fungus. As far as this evidence is concerned, there appears to be no doubt that *Ophiobolus cariceti* can attack wheat and other grasses, but the question that one asks after reading his paper carefully is, What was the condition of the wheat, inoculated or uninocu-

lated? Anyone who has tried to grow wheat in 5-inch pots, as Kirby did, under ordinary greenhouse conditions for considerable periods of time, knows how difficult it is to avoid weak, spindling, under-developed plants, and when one turns to his photograph (Pl. II, fig. *c*) labeled "Healthy and diseased plants after four months' growth," it is noted that the pot of "healthy" plants is so full of drooping, withered, and dead leaves as to make the plants appear decidedly unhealthy. Kirby believes that the fungus also produces a seedling blight[8] for, speaking of field observations, he says (*p. 68*), "An accurate determination of the amount of damage caused by take-all was impossible because many of the plants were killed in the seedling stage." And farther on, under "Symptoms," he says, "The writer has not had the opportunity to study the disease through all its early stages in the field." But what about the early stages in the inoculated pots? Although he inoculated when the seed was sown he notes no Ophiobolus symptoms until ten and a half weeks after inoculation (*p. 74*). His work, therefore, can not be said to present a clear picture of the relationship of attack by *Ophiobolus cariceti* to the condition of the host.

The increase in the percentage of infection on the lime plot in the writers' experiment is in full accord with the work of Kirby and of others, although, as we have already noted, Ophiobolus was able to attack plants growing in acid soil. Of course the relationship of the growth of the wheat to change in acidity of the soil must be considered, for it is now well established (*2*) that growing plants often cause a marked change in the hydrogen-ion concentration of the media in which they are growing.

CONCLUSION

As the results obtained by the writers in controlling this disease involve only one year of experimental work, it would be improper to make any definite recommendations at this time, but inasmuch as these experiments fully confirm numerous other observations made by the writers and by others, there seems to be sufficient ground for concluding that *Ophiobolus cariceti* confines its attack to weakened plants. The discovery of this organism in such widely separated regions as New York, Oregon, Indiana, and Arkansas (*5*) suggests that it is present over a large part of the country, and that it has been overlooked because it is of little economic importance.

SUMMARY

Ophiobolus cariceti was discovered in two wheat fields in Arkansas and on the campus of the state university. Wheat, *Bromus secalinus*, *Chaetochloa geniculata*, *Festuca octoflora*, *Festuca elatior*, and *Hordeum pusillum* were found infected.

Infection appears to be confined to weakened plants. Lack of proper nutrients and water-logged soils in particular were found to be conducive to attacks by this fungus.

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[8] Under unfavorable conditions it is entirely conceivable that seedlings also become susceptible, and McKinney's report[9] of a seedling blight in soil kept at temperatures near 22° and 24° C. would indicate that soil temperatures are important in the development of this disease. From Dickson's work (*1*) it is clear that wheat requires a rather low temperature for good growth. He writes (*p. 849*): "While spring wheat germinated more rapidly at soil temperatures of 24° to 28° C., the germination was more uniform and stronger plants resulted at the lower temperatures—about 8° to 16°. The greatest development of roots as well as tops occurred at the lower soil temperatures. The earliest maturing, most stocky, and best filled plants resulted at soil temperatures of about 16°." As far as winter wheat is concerned, he says: "The cardinal temperatures for the development of Turkey winter wheat were, in general, about 4° C. below those for spring wheat."

[9] McKinney, Harold H. TAKE-ALL AND FOOT-ROT INVESTIGATIONS. *In* U. S. Dept. Agr. Bur. Plant Indus. Cereal Courier, v. 14, p. 24–25. 1922 (mimeographed).

In an experiment involving the use of lime, manure, and commercial fertilizer on different plots of a field in which Ophiobolus had previously been discovered, found attacking a large area of growing wheat, it was found that commercial fertilizer almost completely eliminated the disease, manure decreased the percentage to a considerable degree, and lime increased the incidence of infection.

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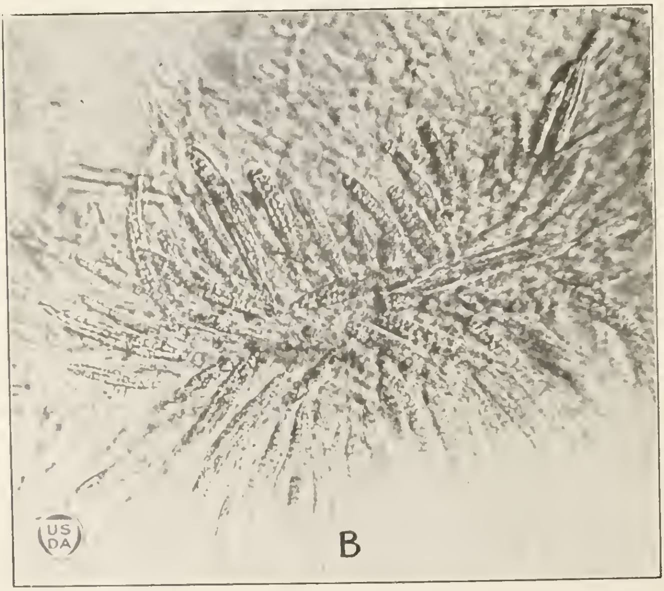
35-81

PLATE 1.

Wheat plant grown on the Pool farm, attacked by Ophiobolus. Note discolored and dead roots and bases of culms and "fuzzy" appearance of roots due to abnormal growth of root hairs. Photographed by senior writer, May 17, 1921.

358

A



B

PLATE 2

A.—Plant attacked by Ophiobolus on the left, healthy plant on the right, all obtained on the Pool farm and photographed at the same time as Plate 1.

B.—Photomicrograph of a cluster of asci of *Ophiobolus cariceti* from *Chaetochloa geniculata.* The diseased host was discovered on the campus of the University of Arkansas. Magnified about 250 times.

PLATE 3

Two views of the Pool field in which Ophiobolus was found over a considerable part of an 11-acre area. Photographed by the senior writer, May, 1921. (Compare with Plate 5.)

33-84

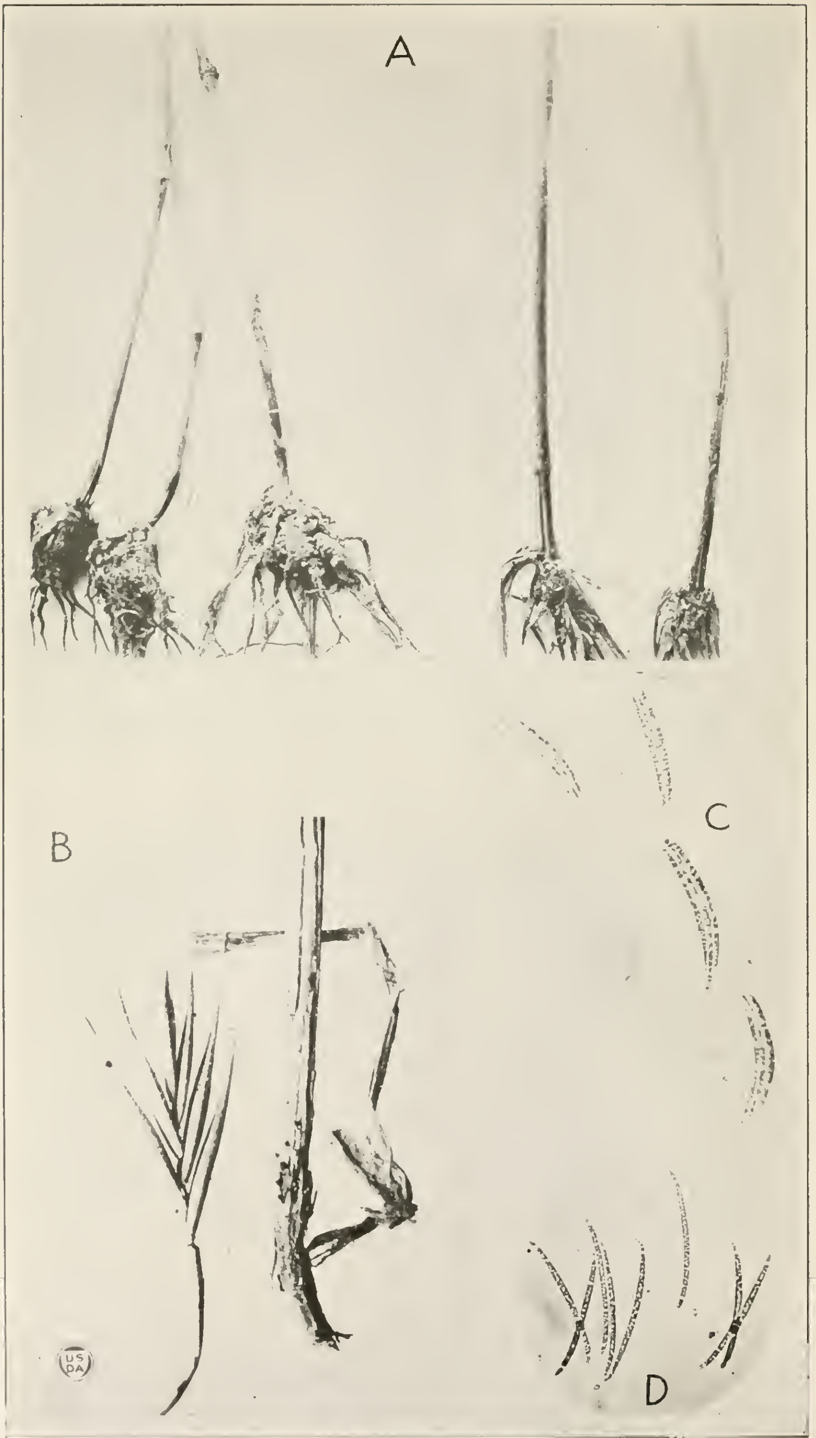

A
B
C
D

PLATE 4

A.—Three plants of *Festuca octoflora* to the left and two of *Hordeum pusillum* to the right, all badly attacked by *Ophiobolus cariceti*. (Slightly enlarged.)

B.—Spikelet of *Festuca octoflora* to the left and lower part of culm to the right, showing perithecial beaks of *Ophiobolus cariceti*. (Slightly enlarged.)

C and D.—Asci and ascospores of *Ophiobolus cariceti* from perithecia found on *Chaetochloa geniculata*. Magnified about 300 times.

PLATE 5

A.—Manure plot to the right, control plot to the left. Photographed April 18, 1922.

B.—Commercial fertilizer plot to the right, lime plot to the left. Photographed April 18, 1922.

C.—Manure plot in early June, 1922. All three photographs by the junior author.


A
B
C

THE PHARYNX AND ALIMENTARY CANAL OF THE HOOKWORM LARVA—NECATOR AMERICANUS[1]

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The following facts, based on observations recently made by the writer (Pl. 1), may be given as reasons for regarding the pharynx of the full-grown larva of *Necator americanus* as somewhat in the nature of a protrusile onchium:

(1) The apex of the pharynx (*on*) in different specimens, fixed and living, varies in position from considerably behind the amphids to a slight protrusion beyond the lips.

(2) The form of the pharynx reminds one of the protrusile onchium of *Mermis*.

(3) The pharyngeal wall is of considerable thickness, such as would impart to it the rigidity necessary for puncturing, while the lumen is very narrow, as in many Tylonchs, "spear-bearing" nemas characterized by the possession of an onchium presumably evolved through conversion of a thin-walled cylindroid or prismoid pharynx into a relatively thick walled, tubular, protrusile spear.

(4) Round the front portion of the pharynx is a refractive ringlike element (*dir on*) similar to that found in nemas armed with a protrusile spear. This encircling element in such cases serves as a guide for the spear when in action. The location, form, and size of this element in *Necator* larvae harmonizes well with the supposition that the associated "onchium" is protrusile; its position accords with the limits of longitudinal motion apparently justly attributable to the "onchium" on the basis of observations made on a considerable number of specimens.

(5) The front part of the pharynx is surrounded (?) by tissue readily explainable as contractile, (*msc*?) similar to that seen in nemas having a protrusile onchium. Such muscles are usually attached to the posterior part of the onchium and to the labial cutin.

(6) The oesophagus of the Necator larva contains "salivary" glands emptying precisely as in a large number of well known nemas possessing a protrusile onchium, namely, three unicellular oesophageal glands having their nuclei located in the posterior oesophageal swelling and emptying forward through three separate ducts, two emptying into the lumen of the oesophagus near its middle, and the third extending farther forward in the dorsal sector of the oesophagus and emptying at the base of the onchium (*gl sal dsl*).

(7) Considered in the light of the known entrance of hookworm larvae into the human host through the skin, these pharyngeal and oesophageal elements fall into a harmonious group in accord with the proposal that the pharynx when acting as a mechanical puncturing organ is assisted by the oesophageal fluid, acting as a solvent, in forcing a passage through the skin.

[1] Accepted for publication May 26, 1923.

A prominent symptom connected with the penetration of hookworm larvae into the human skin is an itching sensation lasting several days. It seems quite as likely that this irritation is caused by a secretion of the larva as by its motions. Reflecting that the "bite" of many insects often, at the time, is accompanied by very little sensation of any kind, while the wound after the withdrawal of the insect's mouth parts becomes inflamed, it is an obvious inference that some substance injected into the wound causes the inflammation rather than the puncture itself. This line of observation and reasoning is in harmony with the supposition that *Necator americanus* works its way through the human cuticle partly by the aid of a solvent, which, incidentally, may account for the accompanying symptoms.

The fact that this onchium of *Necator* larvae has never been seen in action, that is, has never been seen to move in living larvae under the microscope, throws little, if any, doubt on its being protrusile, for it is well established that the examination even of thousands of specimens of nemas of various kinds possessing a protrusile spear may not enable one to make such a direct observation. It is only on rare occasions that the protrusile onchium of a nema has been seen in action, the commonest occasion being its use by the larva in escaping from the egg. Here the larva, even under the blaze and other inhibiting conditions of the microscope, sometimes remains in condition to proceed with its operations. However, many thousands of observations made by numerous competent observers, using hundreds of different species of nemas possessing a protrusile onchium, has resulted in accumulating circumstantial evidence so strong as practically to prove, in this way alone, even unsupported by other evidence, that the onchium is protrusile. This circumstantial evidence, as in the case of *Necator*, relates very largely to differences in the observed position of the onchium, but also relates to its form. On such evidence alone belief that the onchium is protrusile is practically unavoidable, since no other explanation of its form or function can reasonably be offered.

These various considerations appear to me to make it at the very least a reasonable working hypothesis that the pharynx of *Necator americanus*, at the time it reaches the condition of the "full grown larva," is modified into what may fairly be regarded as a protrusile organ.

Interesting morphological speculations with regard to nema anatomy are associated with such an hypothesis. The young larva of *Necator americanus* is said to be rhabitdiform. Now there are certain nema genera in which the rhabditoid form of pharynx, when the species are assembled and suitably arranged, may be "traced" step by step to a pharynx similar to that of *Tylenchus*, a well known genus containing both free-living and parasitic nemas and possessing a protrusile onchium used for piercing, and this fact has led to the promulgation by Marcinowski of a theory that the tylenchoid pharynx may have been evolved from the rhabditoid pharynx.

THE AMPHIDS OF NECATOR

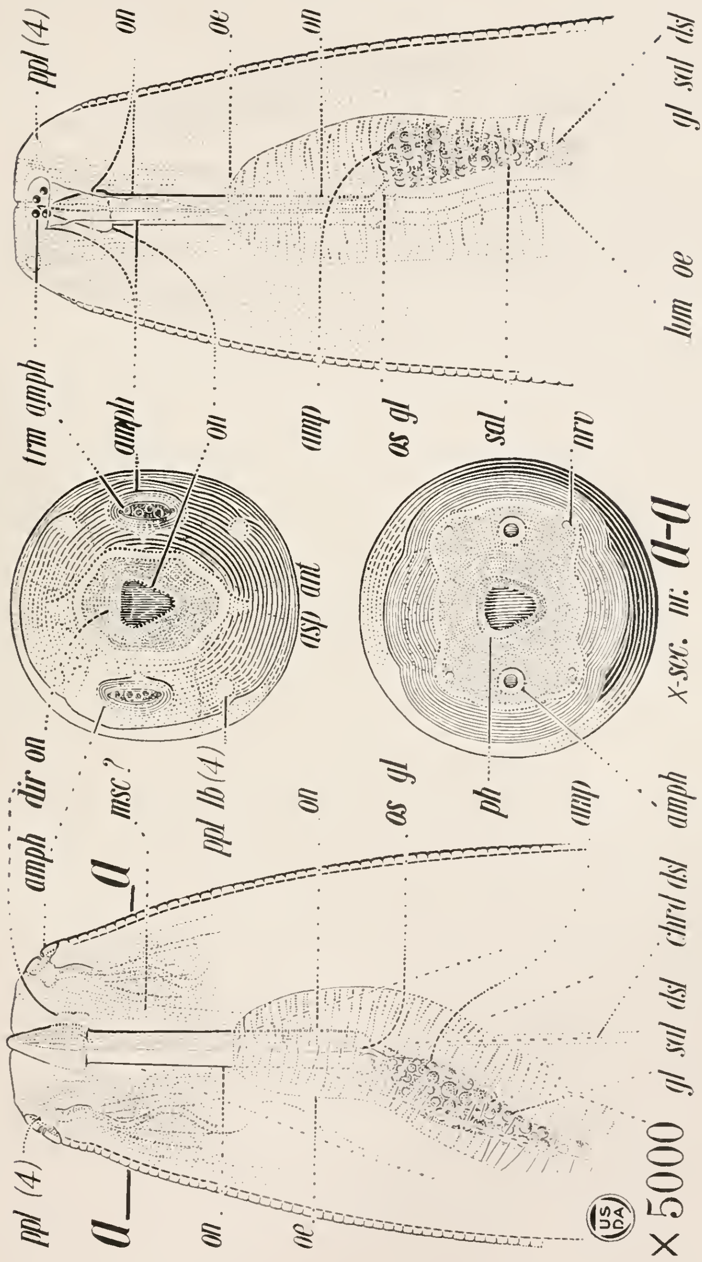
The writer's observations on the amphids on *Necator americanus* (*amph*) are not subject to the qualifications necessary in the case of the pharynx. That these organs on the front of the head of *Necator* fall into the group of organs known as amphids seems to me beyond question. Their number, form, exact symmetry, position, and structure are those typical of well known amphids. The nature of the amphidial

terminals (presumably nerve endings), arranged symmetrically on opposite sides of the head within the amphids (*trm*), lead to the belief that the backward processes connected with them pass to the nerve ring, although they have not been traced thither. It would be an interesting piece of work to trace them backward by means of serial sections of the adult nema.

The cells of the intestine throughout its length, and some of the cells of the body wall, are well stocked, in the young larva, with fat globules. These darken in osmic acid, those in the body wall apparently more vigorously than those in the cells of the intestine, a difference that may be connected with the relative position of the two groups of globules.

PLATE 1

At right and left, dorsal and lateral views of the head of *Necator americanus* free-living larvae when full grown; between them the front view of the head and an optical section near the line *AA*; *ppl*, one of the four labial submedian papillae; *on*, onchium or pharynx; *oe*, oseophagus; *gl sal dsl*, dorsal oesophageal gland; *chrd dsl*, dorsal chord; *amph*, amphid; *amp*, ampulla of the dorsal oesophageal gland; *ph*, pharynx; *os gl*, dorsal oesophageal gland emptying into the lumen of the oesophagus or pharynx; *msc*?, contractile fibers(?); *dir on*, guiding ring encircling the anterior portion of the pharynx; *trm amph*, terminals in the amphid, presumably the end elements of nerve fibers; *sal*, dorsal oesophageal gland; *nrv*, and opposite *AA*, nerve passing to one of the submedian labial papillae; *lum oe*, lumen of the oesophagus. The dorsal and lateral sketches were made from living specimens, the front view and section from specimens fixed in Flemming's solution and mounted in glycerin jelly.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SPECIALIZED VARIETIES OF PUCCINIA GLUMARUM, AND HOSTS FOR VARIETY TRITICI[1]

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INTRODUCTION

Since the discovery of stripe rust in the United States, in 1915, a systematic study of the disease has been conducted by the Office of Cereal Investigations of the Bureau of Plant Industry, United States Department of Agriculture, in cooperation with the Oregon Agricultural Experiment Station, and, later, with the Idaho Agricultural Experiment Station. This paper is the third of a series of contributions to our knowledge of stripe rust in the United States. Former papers (*2, 10*)[3] have dealt with the economic importance and geographical distribution of the disease and with the life history, taxonomy, and morphology of the causative organism. This paper will deal with the known hosts of the rust, our present knowledge of its specialized varieties, and with the comparative susceptibility of certain wheat varieties to *Puccinia glumarum tritici* Erikss. & Henn., as indicated by field and greenhouse experiments.

HOSTS

Eriksson (*4*), in his original report upon *Puccinia glumarum*, when it was separated from the now obsolete *Puccinia rubigo-vera* (DC.) Wint., named wheat, barley, rye, *Elymus arenarius*, and *Agropyron repens* as hosts for the new form. Saccardo (*16, p. 380*) lists the following hosts: *Brachypodium silvaticum, Bromus mollis, Calamagrostis epigeios, Hordeum vulgare, Secale cereale, Triticum caninum, T. compactum, T. dicoccum, T. desertorum, T. distichon, T. durum, T. giganteum, T. polonicum, T. repens.* Hecke (*7*) reports the unpublished work of K. Barfus in which he lists *Dactylis glomerata* as an additional host.

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[2] The writers wish to acknowledge with gratitude the hearty cooperation of Prof. G. R. Hyslop, Mr. J. Allen Clark, and Mr. D. E. Stephens in furnishing wheat varieties for this study, and to thank Mr. C. R. Hursh, Mr. J. T. Bregger, and Mr. J. C. Bell for their aid in taking notes, and Prof. H. P. Barss, Dr. H. B. Humphrey, and Dr. A. G. Johnson for helpful suggestions during the progress of the work and in the preparation of this report. We also wish to express our appreciation for the valuable assistance given by Dr. A. S. Hitchcock and Mrs. Agnes Chase in the determination of the grasses used in the studies herein reported.

[3] Reference is made by number (italic) to "Literature cited," p. 400–401.

Stripe rust has been found in the western part of the United States on wheat, barley, rye, spelt, and emmer in the field, and upon 33 wild grasses. The wild grass hosts found naturally infected include seven species of *Agropyron: A. spicatum* (Pursh) Scribn. & Smith, *A. cristatum* (L.) Gaertn., *A. dasystachyum* (Hook.) Scribn., *A. intermedium* (Host) Beauv., *A. violaceum* (Hornem.) Lange, *A. lanceolatum* Scribn. & Smith; eight species of Bromus: *B. marginatus* Nees, *B. pacificus* Shear, *B. sitchensis* Trin., *B. carinatus* Hook. & Arn., *B. carinatus hookerianus* (Thurb.) Shear, *B. rubens* L., *B. brizaeformis* Fisch. & Mey., and *B. polyanthus* Scribn.; six species of Elymus: *E. canadensis* L., *E. condensatus* Presl, *E. glaucus* Buckl., *E. macounii* Vasey, *E. striatus* Willd., and *E. virginicus* L.; six species of Hordeum: *H. jubatum* L., *H. gussoneanum* Parl., *H. murinum* L., *H. nodosum* L., *H. pusillum* Nutt., and *H. caespitosum* Scribn.; two species of Hystrix: *H. patula* Moench., and *H. californica* (Boland.) Kuntze; three species of Sitanion: *S. jubatum* J. G. Smith, *S. hystrix* (Nutt.) J. G. Smith, and *S. longifolium* J. G. Smith; one species of Phalaris, *P. paradoxa* L., and one wild species of Triticum, *T. aegilops* Beauv.

The rust has been found on wheat in nearly all the western States, but has been reported on barley from only four districts, viz: Eastern Washington, eastern Oregon, western South Dakota, and central California. Only three collections have been made on rye, these being from northern Idaho, eastern Oregon, and western Oregon. The possible significance of the limited distribution of the rust on the last-named hosts will be discussed later.

The occurrence of stripe rust on *Bromus marginatus*, *Elymus canadensis*, *E. glaucus*, *Hordeum nodosum* and *H. jubatum* has been found to be quite general within the known geographic limits of the various grasses and of the parasite. *Hordeum murinum* was very generally infected in 1917 and in 1922 in the lower coast district of California, but has never been found so infected in other sections of the Pacific Coast where this host commonly occurs and where the rust has been present on other grasses. The collections on other hosts have been few in number and indicate that they are not as common hosts for the disease as the others already mentioned. In some cases, at least, they are more limited in their distribution.

As will be shown in a forthcoming paper of this series of studies,[4] a number of collections of stripe rust had been made prior to May, 1915, when stripe rust was first recognized in the United States. These had been identified as several other rusts. Among these collections appear two hosts which have not been found naturally infected since that time. They are *Bromus carinatus hookerianus* (Thurb.) Shear, and *Sitanion longifolium* J. G. Smith.

The variety peculiar to wheat, *Puccinia glumarum tritici* Erikss. and Henn., has been found capable of infecting the following additional hosts when inoculations were made in the greenhouse: *Agropyron tenerum* Vas., and *A. smithii* Rydb.; *Bromus hordeaceus* L., *B. inermis* Leyss., *B. commutatus* Schrad., *B. sterilis* L., *B. tectorum* L., *B. rigidus* Roth, and *B. ciliatus* L.; *Elymus australis* Scribn. and Ball, and *E. robustus* Scribn. and Smith. A detailed discussion of the methods used and the results obtained in connection with the above determinations will be given later under the discussion of specialized races of *P. glumarum*.

[4] In manuscript.

Since the completion of the foregoing studies, headquarters for the investigations on stripe rust have been transferred from Corvallis, Oreg., to Moscow, Idaho, and the research is now being carried on by the senior author and Mr. J. M. Raeder. Since the transfer, the following additional hosts for *Puccinia glumarum tritici* have been determined by greenhouse inoculation: *Agropyron tenerum longifolium* Scribn. and Smith., *A. acutum* (DC.) Roem. and Schult., *Bromus purgans latiglumis* (Scribn.) Shear, *B. lanuginosus* Poir., *B. erectus* Huds., *B. macrostachys* Desf., *B. rigidus* Roth, *B. frondosus* (Shear) Woot. and Standl., *B. richardsonii* Link, *B. adoensis* Hochst., *Hordeum maritimum* Roth, *H. bulbosum* L., *Phalaris canariensis* L., and *Sitanion hystrix* (Nutt.) J. G. S. The grasses in this list, with those listed previously, comprise fifty-nine species of common wild grasses which are known to be hosts for stripe rust as it occurs in the United States. Thirty-three of these have been found naturally infected, and twenty-six of them determined by artificial inoculation with *P. glumarum tritici.* Doubtless other hosts will be added to this list as our knowledge of this rust becomes more complete.

SPECIALIZED VARIETIES

Eriksson (*4*) named five specialized varieties of *Puccinia glumarum*, based on inoculation experiments carried on by him. These were *P. glumarum tritici* on wheat, *P. glumarum hordei* on barley, *P. glumarum secale* on rye, *P. glumarum elymi* on *Elymus arenarius* and *P. glumarum agropyri* on *A. repens.* He states that the varieties on wheat and barley appear to be sharply fixed (scharf fixiert); that is, he could not secure infection upon barley or rye with the variety *tritici*, or upon wheat or rye with the variety *hordei.* The variety *secale* appeared to be less sharply fixed, since he secured slight infection upon wheat inoculated with this variety. He considered that he might have had a mixed culture in the latter case. Neither of the varieties on *Agropyron repens* and on *Elymus arenarius* would infect wheat, barley, or rye, nor was he able in either case to reinfect the original host.

It appears then that Eriksson had good evidence for establishing the varieties *P. glumarum tritici* and *P. glumarum hordei*, but there is some question as to the evidence in the case of the other three varieties.

In the field survey for stripe rust, conducted by the writers, an attempt has been made to record not only the name and location of infected hosts but also the name of other near-by grasses and cereals. It was thought that these notes might furnish valuable data regarding the spread of the rust from host to host in the field and also supplement the study on specialized varieties being conducted in the greenhouse.

As stated above, *Hordeum jubatum* and *Bromus marginatus* are two of the most common hosts for *P. glumarum* in the northwestern States. Wherever these two grasses have been found growing near each other, if one was infected, the other usually was infected also. With few exceptions, wherever infection was common on either of these two wild hosts, stripe rust could be found also on wheat if plants of a susceptible variety could be found growing near by. This also was true to a more limited extent of *Elymus glaucus*, *Hordeum nodosum*, *Elymus canadensis*, and *Elymus condensatus*, although these latter grasses are much more limited in their distribution and do not appear to be as common hosts for stripe rust as are the two first named.

In 1917, and again in 1922, *Hordeum murinum* was found to be one of the most common hosts for stripe rust along the Pacific Coast in southern California. Very seldom was any other host found infected, although other possible hosts often were growing in close proximity.

EXPERIMENTAL METHODS

The greenhouse experiments in the study of specialized varieties of *Puccinia glumarum*, as well as in the testing of wheat varieties for susceptibility to stripe rust, were carried on at Corvallis in a small wing of the greenhouse of the Oregon Agricultural Experiment Station. The methods used in greenhouse inoculations were, with certain modifications, like those employed by Stakman and Piemeisel (*18*) in their stem-rust studies. Every precaution was taken to guard against chance infections.

Seedlings were used with few exceptions, and were inoculated on the first or primary leaf. Plate 1, A and B, shows the method of preparing the seedlings for inoculation and the devices used for obtaining favorable conditions for infection. In every case the plants in a given pot were divided into two groups before any inoculations were made. This was done usually by pulling up or cutting close to the soil those in a line through the center of the pot. A piece of string was then laid on the soil between the two groups of seedlings. One half of these were inoculated and the other half left as control plants. In only one or two instances did any infection develop on the controls, and then only on a plant immediately adjacent to the inoculated ones. In such cases the results were not considered.

Inoculations were made by carefully transferring fresh urediniospores to the upper side of the leaf by means of a small scalpel. Melchers (*13*) found that he could obtain satisfactory infection on wheat with *Puccinia graminis tritici* E. & H. by inoculating the under side of the leaf. This did not prove to be the case with *Puccinia glumarum tritici*. Several trials were made by taking wheat plants of the same age and variety and inoculating part on one side of the leaf and part on the other. Only a very few infections resulted from the inoculations made on the under side, while nearly 100 per cent of those inoculated on the upper side of the leaf became infected.

At first, bell jars and battery jars were used for incubation chambers. Later, however, shallow tubs covered with a window sash were used to good advantage. After inoculation, the pots were placed in a tub containing about 2 inches of water, covered, and left in this moist chamber for 48 hours. They then were removed to the greenhouse bench. The various strains of the rust were kept in separate compartments in the greenhouse, and were isolated by means of partitions made of fine cheesecloth.

RESULTS

Inadequate greenhouse space made it impossible to work with a large number of collections of stripe rust. All of those studied proved to be *Puccinia glumarum tritici*. Each infected wheat readily, rye slightly, barley very slightly, if at all, and oats not at all. Table I shows the results of inoculations made on various varieties of barley and rye with urediniospores from wheat. As barley has been found heavily infected in the field several times, it appears that there must be a distinct special-

ized race which infects that host. Thus far, it has not been possible to obtain the race for experiment from barley.

Under "Character of Infection" the following five grades of infection have been used:

0—No uredinia; flecks and dead areas sometimes present; portions of leaves sometimes killed or discolored (Pl. 3 and 4).

1—Uredinia few or minute, generally surrounded by dead areas; portions of leaves sometimes killed or discolored (Pl. 5).

2—Uredinia normal in appearance, but few and scattered; discoloration of leaf tissues common (Pl. 6, A).

3—Uredinia normal, moderately abundant; little discoloration of leaf tissue (Pl. 6, B).

4—Uredinia normal and very abundant, appearing uniformly over surface of inoculated leaf; no discoloration in early stages of infection (Pl. 6, C).

"Br.," in Table II, indicates that there was pronounced browning of the tissue around the border of the dead areas produced by the rust (See Pl. 3, A).

TABLE I.—*Results obtained when barley and rye varieties were inoculated with urediniospores of stripe rust from wheat*

Crop and variety.	Number of plants.		Character of infection.
	Inoculated.	Infected.	
BARLEY:			
Sandrel	49	0	0
O. A. C. Sel. No. 7	45	0	0
O. A. C. Sel. No. 8	36	0	0
Black Hull-less	48	1	2
Tennessee Winter	33	0	0
White Hull-less	63	0	0
Peruvian	48	0	0
Hannchen	34	2	2
Trebi	68	10	2
RYE:			
Abruzzi	43	6	3
Common	43	12	3

The results of various inoculations made with *Puccinia glumarum tritici* from several sources on various wild grasses and cereals to ascertain which were hosts for the rust are presented briefly in Table II. In some cases the results represent only one trial, while in other instances several experiments were made. Chul wheat was used in all cases.

TABLE II.—*Results of inoculation of urediniospores of Puccinia glumarum tritici from different original and immediate hosts on numerous wild grasses and cereals*

Original host.	Immediate host.	Plant inoculated.	Number of experiments. Infection obtained.	Number of experiments. No infection obtained.	Number of plants. Inoculated.	Number of plants. Infected.	Character of infection.
Wheat	Wheat	*Agropyron cristatum*	1	......	4	3	3
Do	....do	*Agropyron desertorum*	1	......	30	28	4
Do	....do	*Agropyron intermedium*	1	......	18	12	4
Do	....do	*Agropyron smithii*	1	2	45	5	1
Do	....do	*Agropyron spicatum*	1	......	2	2	4
Do	....do	*Agropyron tenerum*	1	1	39	12	2
Do	....do	*Bromus hordeaceus*	1	......	25	2	2
Do	....do	*Bromus inermis*	1	2	74	2	1
Do	....do	*Bromus marginatus*	2	......	42	35	4
Do	....do	*Bromus polyanthus*	2	......	20	15	4
Do	....do	*Bromus secalinus*	......	2	24	0	0
Do	....do	*Dactylis glomerata*	......	4	50	0	0
Do	....do	*Elymus canadensis*	1	......	14	14	4
Do	....do	*Elymus glaucus*	1	......	16	12	4
Do	....do	*Elymus condensatus*	2	......	28	20	4
Do	....do	*Elymus virginicus*	1	......	30	28	4
Do	....do	*Hordeum gussoneanum*	1	......	12	7	4
Do	....do	*Hordeum jubatum*	3	......	50	35	4
Do	....do	*Hordeum nodosum*	2	......	35	20	4
Do	....do	*Hordeum pusillum*	1	......	13	13	4
Do	....do	*Hystrix hystrix*	1	......	13	13	4
Do	....do	*Sitanion jubatum*	1	......	8	7	4
Do	....do	Barley	2	12	235	13	2
Do	....do	Oats	......	4	50	0	0
Do	....do	Rye	3	5	120	20	2
Do	*Bromus marginatus.*	Wheat	3	......	50	40	4
Do	*Elymus canadensis.*	....do	1	......	9	9	4
Do	*Elymus glaucus.*	....do	1	......	9	5	4
Do	*Sitanion jubatum.*	....do	1	......	9	8	4
Bromus marginatus.	Wheat	*Agropyron smithii*	......	1	3	0	0
Do	....do	*Arrhenatherum elatius.*	......	1	22	0	0
Do	....do	*Beckmannia erucaeformis.*	......	1	10	0	0
Do	....do	*Bromus carinatus*	1	1	26	1	1 Br.
Do	....do	*Bromus ciliatus*	1	1	14	2	2
Do	....do	*Bromus hordeaceus*	1	1	40	2	1
Do	....do	*Bromus inermis*	1	1	29	4	1 Br.
Do	....do	*Bromus japonicus*	......	2	25	0	0 Br.
Do	....do	*Bromus pratensis*	......	1	30	2	0 Br.
Do	....do	*Bromus secalinus*	......	1	10	0	0
Do	....do	*Bromus tectorum*	......	1	25	0	0
Do	....do	*Elymus australis*	1	......	20	2	1 Br.
Do	....do	*Elymus canadensis*	1	......	14	5	4
Do	....do	*Elymus glaucus*	1	......	10	3	4
Do	....do	*Festuca elatior*	......	1	20	0	0
Do	....do	Barley	......	2	13	0	0
Do	....do	Wheat	7	......	140	114	4

TABLE II.—*Results of inoculation of urediniospores of Puccinia glumarum tritici from different original and immediate hosts on numerous wild grasses and cereals*—Cont'd.

Original host.	Immediate host.	Plant inoculated.	Number of experiments.		Number of plants.		Character of infection.
			Infection obtained.	No infection obtained.	Inoculated.	Infected.	
Bromus marginatus.	*Bromus marginatus.*	*Bromus marginatus.*	1	......	10	8	4
Do......	.....do.......	*Bromus sterilis*......	1	......	8	8	4
Elymus glaucus.	Wheat.......	*Agropyron caninum*.	......	1	12	0	0
Do.....	.....do.......	*Agropyron repens*...	......	1	10	0	0
Do.....	.....do.......	*Agropyron smithii*..	......	1	13	0	0
Do.....	.....do.......	*Agropyron tenerum*..	......	2	20	0	0 Br.
Do.....	.....do.......	.....do.............	......	1	5	0	0
Do.....	.....do.......	*Beckmannia erucaeformis.*	......	1	11	0	0
Do.....	.....do.......	*Bromus carinatus*...	......	1	30	0	0 Br.
Do.....	.....do.......	*Bromus ciliatus*....	......	1	13	0	0 Br.
Do.....	.....do.......	*Bromus inermis*....	......	2	30	0	0 Br.
Do.....	.....do.......	*Bromus marginatus*.	3	......	42	36	4
Do.....	.....do.......	*Bromus polyanthus*..	1	......	8	4	4
Do.....	.....do.......	*Bromus pratensis*...	......	1	14	0	0 Br.
Do.....	.....do.......	*Bromus secalinus*...	......	2	28	0	0
Do.....	.....do.......	.....do.............	......	1	10	0	0
Do.....	.....do.......	*Bromus sterilis*......	1	......	7	7	3
Do.....	.....do.......	*Bromus vulgaris*....	......	1	12	0	0 Br.
Do.....	.....do.......	*Elymus condensatus*.	1	......	13	10	4
Do.....	.....do.......	.....do.............	1	......	8	4	3
Do.....	.....do.......	.....do.............	1	......	28	14	3
Do.....	.....do.......	*Elymus glaucus*.....	5	......	120	100	4
Do.....	.....do.......	*Elymus robustus*....	1	......	4	1	4
Do.....	.....do.......	*Elymus striatus*....	......	1	8	0	0
Do.....	.....do.......	*Festuca elatior*......	......	1	15	0	0
Do.....	.....do.......	*Festuca pratensis*...	......	1	10	0	0
Do.....	.....do.......	*Hordeum gussoneanum.*	1	......	10	6	3
Do......	.....do.......	*Hordeum murinum*..	2	1	32	10	1
Do.....	.....do.......	.....do.............	2	2	62	8	2
Do.....	.....do..... ..	*Hordeum nodosum*..	2	......	20	18	4
Do.....	.....do.......	*Sitanion jubatum*...	2	......	15	14	4
Do.....	.....do.......	Barley.............	......	4	32	0	0
Do.....	.....do.......	.....do.............	......	1	20	0	0
Do.....	.....do.......	Emmer (Black Winter).	2	......	35	30	4
Do.....	.....do.......	Oats...............	......	1	15	0	0
Do.....	.....do.......	Rye................	......	1	15	0	0
Do.....	.....do.......	Wheat.............	5	......	125	110	4
Hordeum jubatum.	.....do.......	*Agropyron repens*...	......	1	9	0	0
Do.....	.....do.......	*Bromus japonicus*...	......	1	6	0	0 Br.
Do......	.....do	*Bromus polyanthus*..	1	......	19	19	4
Do.....	.....do.......	*Bromus sterilis*......	1	......	6	3	1
Do.....	.....do.......	*Bromus tectorum*....	1	1	23	3	1
Do.....	.....do.......	*Bromus rigidus*.....	1	......	10	2	1 Br.
Do.....	.....do.......	*Elymus glaucus*.....	1	......	12	5	4
Do.....	.....do.......	*Hordeum murinum*..	1	2	20	3	1
Do.....	.....do.......	*Lolium temulentum*.	......	1	13	0	0 Br.
Hordeum nodosum.	.....do.......	*Bromus polyanthus*..	2	......	22	19	4

TABLE II.—*Results of inoculation of urediniospores of Puccinia glumarum tritici from different original and immediate hosts on numerous wild grasses and cereals*—Cont'd.

Original host.	Immediate host.	Plant inoculated.	Number of experiments.		Number of plants.		Character of infection.
			Infection obtained.	No infection obtained.	Inoculated.	Infected.	
Hordeum nodosum.	Wheat	*Sitanion jubatum*	2		26	24	4
Do	do	Rye		1	18	0	0
Do	do	Wheat	2		42	30	4
Do	*Bromus polyanthus.*	*B. carinatus*		2	46	0	0 Br.
Do	*Hordeum nodosum.*	*Elymus condensatus.*	1		12	8	3
Do	do	*Bromus commutatus.*		1	20	0	0
Do	do	*Hordeum nodosum*	1		12	12	4
Do	do	*Dactylis glomerata*		5	60	0	0

The list in Table II includes 48 species of wild grasses which have been shown to be hosts of *P. glumarum tritici.* It includes 19 species of Bromus, 11 species of Agropyron, 7 species of Elymus, and 1 species each of Hystrix, Sitanion, and Phalaris. Collections have been made in the field on 12 additional infected grass hosts, but it has not been possible to determine if they were infected with the race which goes to wheat. These 12 species include: *Agropyron inerme* (Scribn. and Sm.) Rydb., *A. dasystachyum* (Hook.) Scribn., *A. caninum*, *Bromus pacificus* Shear, *Bromus sitchensis* Trin., *Bromus carinatus hookerianus* (Thurb.) Shear, *Bromus brizaeformis* Fisch. and Mey., *Hordeum caespitosum* Scribn., *Hystrix californica* (Boland.) Kuntze, *Elymus striatus* Willd., *Elymus macounii* Vasey, *E. triticoides* Buckl., *Phalaris paradoxa* L., *Sitanion hystrix* (Nutt.) J. G. Smith, *Sitanion longifclium* J. G. Smith, and *Aegilops cylindrica* Beauv.

Only one specialized variety apparently has been used in these inoculation experiments, and this appears to be *Puccinia glumarum tritici* Erikss. & Henn. There are some indications that at least one other variety is present in the United States. This probably is *Puccinia glumarum hordei* Erikss. & Henn., as indicated by the fact that barley has been found heavily infected in a few instances, while the race commonly found on wheat does not pass readily to barley. There also is evidence that the rust on *Hordeum murinum* may not be the variety commonly found on wheat. A discussion of these varieties is reserved for a later paper.

In only one case in Table II does there occur a difference of more than two in the grade of infection recorded when the same host was inoculated with the rust taken from the different hosts. When *Bromus sterilis* was inoculated with rust spores from *Hordeum jubatum* the infection was recorded as 1. When spores were taken from *Bromus marginatus* and from *Elymus glaucus* the result was a grade of 4 in each case. There are two possible explanations for these results. There may have been two strains of *Puccinia glumarum tritici* involved, or it is possible that there were two strains of *Bromus sterilis* used, as the seed came from

two different sources. During the course of this work there have been several indications that there may be strains of the specialized variety *Puccinia glumarum tritici*, the same as exist in *P. graminis tritici*, as shown by Stakman and Piemeisel (*18*). During the course of these studies evidence has accumulated indicating the possible existence of varieties or strains of various grasses which may react very differently to a given strain of the parasite. This phase of the question is under investigation.

SUSCEPTIBILITY OF WHEAT VARIETIES TO PUCCINIA GLUMARUM TRITICI

No attempt will be made here to review the very extensive literature upon the subject of rust resistance. Complete reviews of the subject from various angles have been made by Eriksson and Henning (*5*), Biffen (*1, p. 40–44*), Comes (*3*), Nilsson-Ehle (*14*), and, more recently, Henning (*8*). The study in Europe on the relative resistance of various wheats to *Puccinia glumarum* also has been extensive. As might have been foreseen, the results obtained have varied greatly, for the men engaged have worked under entirely different conditions, and undoubtedly with different strains of the varieties of wheat used as well as with different strains of the rust.

This paper summarizes the results obtained through study of a large number of varieties of wheat for resistance to *Puccinia glumarum tritici* as it occurs in western Oregon. The wheat varieties were obtained partly from the Office of Cereal Investigations and partly from the Department of Farm Crops of the Oregon Agricultural College. Every precaution has been taken to keep these varieties free from mixtures.

Both greenhouse and nursery studies were carried on at Corvallis, Oreg. In all, 337 varieties and strains of wheat were grown in the rust nursery. Only 163 of these are included in Table III. The remainder were grown in only one year and therefore are not included. Ninety-two varieties were studied under greenhouse conditions. Not all of the 163 varieties were sown in the nursery in each of the three seasons through which the experiments were extended. In 1918 only 72 varieties and strains were grown. In 1919 additional seed was obtained and 142 varieties and strains were grown. Still other varieties were added in 1920. Likewise, not all of the 92 varieties were grown in the greenhouse in any one season. Because of inadequate space and assistance it was impossible to study in the greenhouse all of the varieties that were used in the rust nursery.

NURSERY EXPERIMENTS

In the rust nursery the varieties were all sown in rod rows. In some cases replicated sowings were made; in other cases there was only one row of each variety. All varieties of which seed was available were sown in the fall regardless of whether they were of winter or spring habit. Under the climatic conditions prevailing at Corvallis it was found that spring varieties, when sown in the autumn, survived the winter in practically as good condition as the winter varieties. In the first two years such varieties as were known to be of spring habit and of which seed was available were sown in duplicate rows in the spring. In 1920 all varieties of which seed was available were sown again in duplicate rows in the

spring regardless of whether they were winter or spring wheats. Of course the winter varieties did not head when sown in the spring nursery, so that it was impossible to get notes on head infection.

The methods of making inoculations in order to induce an epiphytotic of stripe rust in the nursery were such as seemed to offer the best conditions possible for an abundant and widespread infection. Border rows of Chul, one of the most susceptible varieties of wheat, were sown around all of the plots. These border rows were inoculated both by spraying a suspension of urediniospores over their entire length and by hand inoculation of individual plants at intervals in them.

In addition to the border-row inoculations, one end of each varietal row was sprayed with a spore decoction and individual plants in every row were hand inoculated. Hand inoculations were made by smearing spores on the leaves with a scalpel, spraying the plants with water, and covering the inoculated plants with inverted flower pots for a period of 48 hours. The quantity of infection on the border row served as a control, showing whether or not climatic and other conditions were favorable for abundant infection.

The results of the 3-year study of the effects of such inoculations on 163 varieties of common, club, poulard, durum, and Polish wheats, and emmer, spelt, and einkorn, when grown in the nursery, are shown in Tables III and IV.

The method used to indicate the quantity of infection recorded in Table IV needs a word of explanation. It seemed to the writers that the usual method of estimating the quantity of rust on cereals was not the best for accurately indicating the quantity of stripe rust present. It was found that varieties differed widely in the proportion of the plants in the row which showed infection, as well as in the quantity of rust on those plants which were rusted. In other words, there was much variation in the rapidity and extent of the spread of the rust on the different varieties.

Table III shows, for each variety, the proportion of the plants which were rusted at the Oregon Agricultural Experiment Station in the fall of 1917 and the spring of 1918, expressed in percentages, and the degree of rustiness of these infected plants, expressed in terms of a scale ranging from 1 to 10. The degree of infection refers to the proportion of plant surface covered with rust. This necessitates the recording of two numbers to indicate the comparative susceptibility of each variety. In order to reduce these figures to a single product which would express at a glance this comparative susceptibility, the following plan was devised. The product of the percentage of plants infected by the degree of infection would give in comparative terms the average infection of any given variety, with a possible maximum of 1,000, where 100 per cent of the plants in the row were infected with a maximum degree of 10. This method of computation has been used to indicate infection of the plants when in leaf and when heading. Infection data were taken when about 5 leaves were out, and again when the heads were well out of the boot.

As an example of the application of this method, take from Table III the data for Chul when grown as a winter wheat in 1917–18. The percentages of infected plants recorded for the first, second, and third replications are 80, 100, and 70, respectively, and the degrees of infection are 6, 6, and 3, respectively. The sum of the products of each per-

centage by the corresponding degree is 1,290. Dividing this sum by 3, the number of replications, gives 430, which, therefore, is the average product representing the comparative susceptibility of Chul wheat, based on a possible maximum of 1,000 (Table IV).

In Table III, the percentage of plants infected and the degree of infection are shown for the 1917–18 season. The average susceptibility of each variety is shown in Table IV, not only for the first season, but for the seasons 1918–19 and 1919–20 as well.

Evidence of the varying susceptibility, even of strains within a variety, is shown in the case of the White Winter variety. In Table IV are listed five different C. I. numbers [5] of this variety. It will be noted that C. I. No. 5219 is somewhat susceptible, while three other strains are entirely immune, so far as those experiments show. The White Winter selection, C. I. No. 5222, which is quite susceptible, is also morphologically distinct from the other strains. These results emphasize the statement made by Vavilov (*19*) that workers should be careful to designate the exact botanical classification of host as well as parasite. Vavilov suggests that pure lines are desirable in studies of varietal susceptibility.

[5] Accession numbers of the Office of Cereal Investigations.

TABLE III.—*Data obtained on the percentage of plants infected and the degree of infection by Puccinia glumarum tritici on varieties of wheat, emmer, spelt, and einkorn at the fifth-leaf stage and at maturity in the fall of 1917 and the spring of 1918, at the Oregon Agricultural Experiment Station*

COMMON WHEATS

Group and variety.	C. I. No.	Fall sown.				Spring sown.			
		5th-leaf stage.		Heading.		5th-leaf stage.		Heading.	
		Plants infected, per cent.	Degree of infection.	Plants infected, per cent.	Degree of infection.	Plants infected, per cent.	Degree of infection.	Plants infected, per cent.	Degree of infection.
Hard red spring:									
Barletta (Minn. 1148)	3297	...	...	...	...	2	1	0	0
Cedar	4117	...	...	...	...	60	6	0	0
Chul	2406	80	6	95	5	90	7	T.	0
		100	6	100	4	75	7	T.	0
		70	3	85	4	80	7	0	0
Early Red Fife	4932	50	5	0	0	...	...	...	...
		30	5	0	0	...	...	...	...
		0	0	0	0	...	...	...	...
Ghirka (Ghirka Spring)	1517	...	...	...	...	100	8	0	0
						80	8	0	0
						100	7	0	0
Haynes Bluestem	2874	0	0	0	0	70	4	0	0
		0	0	0	0	40	6	0	0
		0	0	0	0	15	4	0	0
Kinney	5197	0	0	0	0	...	...	...	...
		0	0	0	0	...	...	...	...
		0	0	0	0	...	...	...	...
Kinney (Surprise)	5197	10	1	0	0	...	...	...	...
		50	1	0	0	...	...	...	...
		10	1	0	0	...	...	...	...
Koola	2203	80	3	0	0	100	8	0	0
		60	5	10	2	80	7	0	0
		80	7	0	0	100	7	0	0

Marquis	3641	0	0	0	0	50	5	0	0
		0	0	0	0	70	5	0	0
		0	0	0	0	100	8	0	0
Pioneer	4324	20	1	T.	T.	10	6	0	0
		0	0	0	0	20	6	0	0
		0	0	0	0	60	8	0	0
Preston	3081	30	3	0	0	40	5	0	0
		2	4	0	0	30	3	0	0
		0	0	0	0	15	4	0	0
Hard red winter:									
Kanred	5146	0	0	0	0	80	6	0	0
		0	0	0	0	100	6	0	0
Kharkof	2193	0	0	0	0	...	...	...	...
		0	0	0	0	...	...	...	...
Turkey	1558	0	0	0	0	...	...	...	...
		0	0	0	0	...	...	...	...
		0	0	0	0	...	...	...	...
Soft red winter:									
Banner Berkeley	...	0	0	0	0	...	...	...	...
Fulcaster	3406	0	0	0	0	...	...	...	...
Fultz	3416	0	0	0	0	...	...	...	...
Gipsy (Farmers Friend)	5793	...	...	...	...	...	7	0	0
Golden Cross	5180	6	1	T.	2	...	...	...	...
		0	0	0	0	...	...	...	...
		0	0	0	0	...	...	...	...
Grandprize	4876	40	3	0	0	...	...	...	...
		80	6	0	0	...	...	...	...
		50	5	0	0	...	...	...	...
Harvest Queen	5314	10	1	T.	T.	...	...	...	...
		0	0	0	0	...	...	...	...
		0	0	0	0	...	...	...	...
Nebraska No. 28	5147	20	2	20	1	...	...	...	...
		0	0	0	0	...	...	...	...
Red Russian	4509	10	2	0	0	...	...	...	...
		20	2	0	0	...	...	...	...
		0	0	0	0	...	...	...	...
Zimmerman	2907	0	0	0	0	...	...	...	...

TABLE III.—*Data obtained on the percentage of plants infected and the degree of infection by Puccinia glumarum tritici on varieties of wheat, emmer, spelt, and einkorn at the fifth-leaf stage and at maturity in the fall of 1917 and the spring of 1918, at the Oregon Agricultural Experiment Station*—Continued

COMMON WHEATS—continued

Group and variety.	C. I. No.	Fall sown.				Spring sown.			
		5th-leaf stage.		Heading.		5th-leaf stage.		Heading.	
		Plants infected, per cent.	Degree of infection.	Plants infected, per cent.	Degree of infection.	Plants infected, per cent.	Degree of infection.	Plants infected, per cent.	Degree of infection.
White:									
Baart (Early Baart)	1697	50	2	80	3	100	8	0	0
		80	6	90	4	100	7	0	0
		20	3	15	2	100	8	0	0
Challenge (Webbs Challenge White)	4683	0	0	0	0	.....	.....	.....	.....
		0	0	0	0	.....	.....	.....	.....
		0	0	0	0	.....	.....	.....	.....
Dicklow	4758	0	0	0	0	10	2	.....	.....
						0	0	0	0
						0	0	.....	.....
Eaton	4682	0	0	0	0	.....	.....	.....	.....
		0	0	0	0	.....	.....	.....	.....
		0	0	0	0	.....	.....	.....	.....
Federation	4734	20	3	15	2	90	7	0	0
		10	3	0	0	75	7	0	0
		50	2	0	0	100	7	.....	.....
Florence	4170	.....	.....	.....	.....	50	4	0	0
						50	7	0	0
						20	6	0	0
Foisy	5253	0	0	0	0	.....	.....	.....	.....
		50	2	0	0	.....	.....	.....	.....
		20	3	0	0	.....	.....	.....	.....
Goldcoin (American Banner)	3016	20	2	0	0	.....	.....	.....	.....
		10	2	0	0	.....	.....	.....	.....

Goldcoin (Fortyfold) (Wash. 351)	6176	20	2	0	0	...	...	...	...
		40	2	0	0	...	...	...	...
		20	3	0	0	...	...	...	...
Jumbuck	4608	...	...	...	...	50	6	0	0
Pacific Bluestem	5231	...	...	...	...	100	8	0	0
						100	8	0	0
						100	8	0	0
Rink	5868	0	0	0	0	...	...	...	...
		0	0	0	0	...	...	...	...
		0	0	0	0	...	...	...	...
Royalton (Minn. 1037)	4968	...	...	...	...	0	0	0	0
Sonora	3036	0	0	0	0	...	...	...	...
		T.	T.	0	0	...	...	...	...
		0	0	0	0	...	...	...	...
Talimka	2495	100	5	100	5	100	8	T.	T.
		60	4	100	4	95	8	0	0
		50	4	40	2	100	8	0	0
White Winter	4684	0	0	0	0	...	...	...	...
		0	0	0	0	...	...	...	...
		0	0	0	0	...	...	...	...
Wilhelmina	4193	30	4	0	0	...	...	...	...
		0	0	0	0	...	...	...	...
		0	0	0	0	...	...	...	...
Miscellaneous:									
Hansia Broach	4690	100	9	100	3	100	9	50	5
		100	8	100	8	95	9	T.	T.
		100	7	100	5	100	8	T.	T.
Popatia Nadiad	4696	100	10	70	10	100	10	75	10
		100	9	100	8	100	9	T.	T.
		100	7	100	8	100	8	T.	T.
Tigharia	4564	...	...	...	...	100	7	50	2
						100	8	0	0
						100	8	0	0
Western Sweepstakes	5607	0	0	0	0	...	...	...	...

TABLE III.—*Data obtained on the percentage of plants infected and the degree of infection by Puccinia glumarum tritici on varieties of wheat, emmer, spelt, and einkorn at the fifth-leaf stage and at maturity in the fall of 1917 and the spring of 1918, at the Oregon Agricultural Experiment Station*—Continued

CLUB WHEATS

Group and variety.	C. I. No.	Fall sown.				Spring sown.			
		5th-leaf stage.		Heading.		5th-leaf stage.		Heading.	
		Plants infected, per cent.	Degree of infection.	Plants infected, per cent.	Degree of infection.	Plants infected, per cent.	Degree of infection.	Plants infected, per cent.	Degree of infection.
Dale (Dale Gloria)	4231	70	5	15	1	100	8	....	....
		20	2	0	0	80	9	....	....
		60	3	8	2	95	8	0	0
Hybrid 128	4512	0	0	0	0	....	....	....	....
		5	2	0	0	....	....	....	....
		0	0	0	0	....	....	....	....
Hybrid 143	4160	0	0	0	0	....	....	....	....
		0	0	0	0	....	....	....	....
		0	0	0	0	....	....	....	....
Little Club	4219	40	5	10	1	....	....	....	....
		25	4	0	0	....	....	....	....
		60	3	3	1	....	....	....	....
Salt Lake Club	3018	20	3	15	2	....	....	....	....
		85	4	60	2	....	....	....	....
		75	4	60	3	....	....	....	....
POULARD WHEAT									
Titanic	5535	0	0	0	0	....	....	....	....
		0	0	0	0	....	....	....	....
		10	2	0	0	....	....	....	....

DURUM WHEATS									
Acme	5284	...	...	...	...	100	8	0	0
						100	8	0	0
						100	7	0	0
Arnautka	4064	10	1	0	0	50	6	0	0
		10	2	0	0	90	8	0	0
						30	8	0	0
Gharnovka	1443	...	...	...	...	100	7	0	0
						90	6	0	0
Iumillo	1736	10	2	0	0	70	8	0	0
		0	0	0	0	100	8	0	0
		0	0	0	0	90	5	0	0
Kubanka No. 8	4063	10	2	0	0	50	6	0	0
		0	0	0	0	20	6	0	0
		0	0	0	0	50	7	0	0
Marouani	2235	...	...	...	...	100	7	0	0
						100	8	0	0
Mindum (Minn. 470)	5296	...	...	...	...	100	8	0	0
EMMER									
Brown Winter	2483	0	0	0	0	...	...	...	...
Khapli	4013	...	...	...	...	60	6	0	0
						80	7	0	0
Vernal (White Spring) (Minn. 1165)	3686	...	...	...	...	10	7	0	0
Vernal (White Spring)	1522	...	...	...	...	100	8	0	0
Do.	1524	...	...	...	...	100	8	0	0
						90	7	0	0
						100	8	0	0
Do.	4781	...	...	...	...	90	8	0	0
						100	8	0	0

TABLE III.—*Data obtained on the percentage of plants infected and the degree of infection by Puccinia glumarum tritici on varieties of wheat, emmer, spelt, and einkorn at the fifth-leaf stage and at maturity in the fall of 1917 and the spring of 1918, at the Oregon Agricultural Experiment Station*—Continued

Group and variety.	C. I. No.	Fall sown.				Spring sown.			
		5th-leaf stage.		Heading.		5th-leaf stage.		Heading.	
		Plants infected, per cent.	Degree of infection.	Plants infected, per cent.	Degree of infection.	Plants infected, per cent.	Degree of infection.	Plants infected, per cent.	Degree of infection.
SPELT									
Red Winter	1772	0	0	0	0	....	....	....	....
POLISH WHEAT									
Polish	5524	....	....	....	....	50 50 50	6 8 7	0 0 0	0 0 0
EINKORN									
Einkorn	2433	....	....	....	....	0	0	0	0

TABLE IV.—*Data obtained on the susceptibility of varieties of wheat, emmer, spelt, and einkorn to infection by Puccinia glumarum tritici as determined by records taken when the plants, grown in the nursery, were developing the fifth leaf and when they were heading, in one or more of the three years from 1918 to 1920, inclusive, at the Oregon Agricultural Experiment Station, Corvallis, Oreg.*

COMMON WHEATS

Group and variety.	C. I. No.	Varietal susceptibility.											
		1917–1918				1918–1919				1919–1920			
		Fall sown.		Spring sown.		Fall sown.		Spring sown.		Fall sown.		Spring sown.	
		5-leaf stage.	Heading.	5-leaf stage.	Heading.	5-leaf stage.	Heading.	5-leaf stage.	Heading.	5-leaf stage.	Heading.	5-leaf stage.	Heading.
Hard red spring:													
Barletta (Minn. 1148)	3297	......	......	2	0	0	0	......	......	0	0	0	0
Cedar	4117	......	......	360	0	0	0	......	......	0	0	T.	0
Chul (Alturas, Calif.)	2227	......	......	......	......	293	594	......	......	0	T.	40	0
Chul	2227	......	......	......	......	133	792	......	......	0	0	30	0
Chul (Yantagbay)	2404	......	......	......	......	135	700	......	......	T.	0	160	75
Chul	2406	430	405	572	T.	830	792	125	0	50	T.	360	0
Early Red Fife	4932	133	0	......	......	0	0	......	......	0	0	0	0
Ghirka (Ghirka Spring)	1517	......	......	713	0	0	15	50	0	0	0	3	0
Haynes Bluestem	2874	0	0	193	0	0	0	3	0	0	0	0	0
Humpback	3690	......	......	......	......	35	0	......	......	0	0	0	0
Huron	3315	......	......	......	......	0	0	......	......	0	0	0	0
Kinney	5197	0	0	......	......	T.	0	......	......	0	0	0	0
Kinney (Surprise)	5197	23	0	......	......	20	20	......	......	0	0	80	0
Koola	2203	367	7	687	0	60	60	425	0	1	0	250	0
Marquis	3641	0	0	467	0	5	5	1	0	0	0	T.	0
Marquis (Minn. 1239)	3641	......	......	......	......	T.	0	......	......	0	0	T.	0
Pioneer	4324	7	T.	220	0	0	0	1–	0	0	0	T.	0
Preston (Minn. 924)	2958	......	......	......	......	0	0	......	......	0	0	T.	0
Preston	3081	33	0	117	0	0	0	1–	0	0	0	0	0

TABLE IV.—*Data obtained on the susceptibility of varieties of wheat, emmer, spelt, and einkorn to infection by Puccinia glumarum tritici as determined by records taken when the plants, grown in the nursery, were developing the fifth leaf and when they were heading, in one or more of the three years from 1918 to 1920, inclusive, at the Oregon Agricultural Experiment Station, Corvallis, Oreg.*—Continued

COMMON WHEATS—continued

Group and variety.	C. I. No.	Varietal susceptibility.											
		1917–1918				1918–1919				1919–1920			
		Fall sown.		Spring sown.		Fall sown.		Spring sown.		Fall sown.		Spring sown.	
		5-leaf stage.	Heading.	5-leaf stage.	Heading.	5-leaf stage.	Heading.	5-leaf stage.	Heading.	5-leaf stage.	Heading.	5-leaf stage.	Heading.
Hard red winter:													
Kanred	5146	0	0	540	0	0	0	...	...	0	0	75	...
Kharkof	2193	0	0	...	...	0	0	...	...	T.	0	50	0
P-1066 (Kansas)	5879	...	...	...	...	8	30	...	...	0	0	140	...
P-1068 (Kansas)	5880	...	...	...	...	0	0	...	...	0	0	90	...
Turkey	1558	0	0	...	...	100	10	...	...	0	0	5	0
Soft red winter:													
Banner Berkeley	...	0	0	...	...	25	0	50	0	0	0	0	...
Fulcaster	3406	0	0	...	...	35	0	160	0	0	0	25	...
Fultz	3416	0	0	...	...	60	0	10	0	0	0	T.	...
Fultz (Economy)	3397	...	...	...	...	315	285	...	...	180	0	800	...
Gipsy (Farmers Friend)	5793	...	...	...	0	...	...	...	...	...	...	...	...
Golden Cross	5180	2	1	0	0	53	500	...	...	0	0	0	...
Grandprize	4876	283	0	...	...	681	250	...	...	T.	0	760	0
Harvest Queen	5314	3	T.	...	...	T.	0	...	...	0	0	T.	0
Illini Chief	5406	T.	...	...	...	78	10	160	0	0	0	100	...
Jones Fife	3452	...	...	...	...	471	100	...	...	1	0	250	...
Jones Fife	4468	...	...	...	...	405	100	...	...	1	0	175	...
Jones Fife	4468	...	...	...	...	228	60	...	...	2	0	300	...
Jones Fife (Super)	5544	...	...	...	...	384	50	446	0	50	0	570	...
Jones Fife	5608	...	...	...	...	340	25	...	...	10	0	350	...
Jones Fife	6177	...	...	...	...	80	0	...	...	80	0	800	...

Mealy	3358	...	...	...	...	0	0	...	...	0	0	0	0
Nebraska No. 28	5147	20	10	...	...	33	10	...	...	T.	0	475	0
Ohio Hybrid 700	5378	...	...	...	...	493	100	...	...	95	0	700	...
Ohio Hybrid 703	5379	...	...	...	...	373	300	...	...	95	0	800	...
Prosperity	5937	...	...	...	...	597	75	...	...	190	0	800	...
Prosperity (American Bronze)	5638	...	...	...	...	382	90	...	...	75	0	270	...
Purdue No. 1	4871	...	...	...	...	850	700	...	...	600	0	950	...
Quadruple (Wash. 601)	6466	...	...	...	...	0	0	...	...	T.	0	25	...
Red Russian	4509	20	0	...	...	5	T.	...	...	0	0	0	...
Red Russian (Wash. 270)	4509	...	...	...	...	0	0	...	...	0	0	0	...
Ruddy	6465	...	...	...	...	0	0	...	...	0	0	0	...
Stoner	2980	...	...	...	...	305	270	...	...	1	0	475	...
Triplet (Wash. 597)	5408	...	...	...	...	T.	0	...	...	0	0	0	0
Zimmerman	2907	0	0	...	...	45	0	T.	0	0	0	30	...
White:													
Allen (Red Allen)	5407	...	...	...	...	25	40	...	...	0	0	40	0
Arcadian (Early Arcadia)	4220	...	...	...	...	325	160	...	...	80	0	700	...
Do	5536	...	...	...	...	498	400	...	...	170	0	800	...
Do	5940	...	...	...	...	488	500	...	...	75	0	686	...
Baart (Early Baart)	1697	213	210	767	0	195	70	375	20	50	0	300	0
Challenge (Webbs Challenge White)	4683	0	0	...	...	T.	0	...	...	0	0	...	...
Dicklow	4758	0	0	7	0	30	T.	75	0	0	0	0	0
Eaton	4682	0	0	...	...	3	0	...	...	0	0	100	0
Federation	4734	63	10	618	0	99	3	...	...	T.	0	570	0
Florence	4170	...	...	223	0	475	10	...	...	0	0	T.	0
Foisy	5253	53	0	...	...	1	0	...	...	0	0	50	0
Genesee Giant	1744	...	...	...	...	...	...	...	...	200	0	...	...
Goldcoin (American Banner)	3016	30	0	...	...	520	700	450	0	T.	0	600	...
Goldcoin (Fortyfold)	4156	...	...	...	...	270	0	...	...	0	0	180	0
Goldcoin (Fortyfold) (Wash. 351)	6176	60	0	...	...	25	0	...	...	0	0	250	...
Jumbuck	4608	...	...	300	0	0	0	...	...	0	0	T.	0
Kofod	2997	...	...	...	...	0	0	...	...	0	0	0	0
Link (Missing Link)	4866	...	...	...	...	38	100	...	...	0	0	140	0
Martin (Martin Amber)	5925	...	...	...	...	38	20	...	...	T.	0	350	...
Pacific Bluestem	5231	...	...	800	0	155	35	130	0	0	0	20	0
Prohibition	5223	...	...	...	...	45	0	...	...	T.	0	20	0
Rink	5218	...	...	...	...	0	0	...	...	0	0	0	...
Rink	5868	0	0	...	...	T.	T.	...	...	0	0	T.	0
Royalton (Minn. 1037)	4968	...	...	0	0	0	0	...	...	0	0	0	0

TABLE IV.—*Data obtained on the susceptibility of varieties of wheat, emmer, spelt, and einkorn to infection by Puccinia glumarum tritici as determined by records taken when the plants, grown in the nursery, were developing the fifth leaf and when they were heading, in one or more of the three years from 1918 to 1920, inclusive, at the Oregon Agricultural Experiment Station, Corvallis, Oreg.*—Continued

COMMON WHEATS—continued

Group and variety.	C. I. No.	Varietal susceptibility.											
		1917–1918				1918–1919				1919–1920			
		Fall sown.		Spring sown.		Fall sown.		Spring sown.		Fall sown.		Spring sown.	
		5-leaf stage.	Heading.	5-leaf stage.	Heading.	5-leaf stage.	Heading.	5-leaf stage.	Heading.	5-leaf stage.	Heading.	5-leaf stage.	Heading.
White—Continued.													
Satisfaction (Smith's Rustproof)	3588	......	......	......	......	110	240	......	......	0	0	140	......
Sonora	3036	T.	0	......	......	25	0	......	......	T.	0	T.	0
Do	4293	......	......	......	......	0	T.	......	......	0	0	0	0
Do	4501	......	......	......	......	0	0	......	......	0	0	0	0
Talimka	2495	313	327	787	T.	289	490	136	150	T.	0	200	95
White Winter	4684	0	0	......	......	......	......	......	......	......	......	......	......
Do	5219	......	......	......	......	33	5	......	......	T.	0	200	0
Do	5224	......	......	......	......	0	0	......	......	0	0	0	......
Do	5232	......	......	......	......	0	0	......	......	0	0	0	......
White Winter selection	5222	......	......	......	......	206	360	......	......	5	0	800	0
Wilhelmina	4193	40	0	......	......	0	0	......	......	0	0	0	0
Winter Bluestem	5409	......	......	......	......	0	T.	......	......	0	0	0	......
Miscellaneous:													
Hansia Broach	4690	800	533	852	83	750	900	230	300	60	0	150	50
Turkey x Chul (F_3, No. 1)	......	......	......	......	......	3	100	......	......	T.	0	480	0
Do. (F_3, No. 2)	......	......	......	......	......	270	190	......	......	0	0	350	0
Do. (F_3, No. 3)	......	......	......	......	......	35	140	......	......	0	0	420	0
Little Joss	6730	......	......	......	......	0	0	......	......	0	0	0	......
Popatia Nadiad	4696	867	557	900	250	900	800	580	600	250	T.	800	300
Tigharia	4564	......	......	767	33	738	150	......	......	......	......	......	......
Trimenia	2443	......	......	......	......	0	0	......	......	0	0	T.	......
Western Sweepstakes	5607	0	0	......	......	T.	0	......	......	0	0	280	......

CLUB WHEATS

Coppei	4238	......	......	......	......	5	0	......	......	T.	0	285	0
Dale (Dale Gloria)	4231	190	20	760	0	693	570	......	......	20	0	550	0
Hybrid 60	5024	......	......	......	......	0	0	......	......	0	0	T.	0
Hybrid 123	4511	......	......	......	......	5	0	......	......	1	0	30	0
Hybrid 128	4512	......	......	......	......	0	0	......	......	0	0	T.	......
Hybrid 128	4512	3	0	......	......	48	20	......	......	T.	0	270	0
Hybrid 143	4160	0	0	......	......	0	0	0	0	0	0	T.	0
Hybrid 143	4513	......	......	......	......	0	0	175	0	T.	0	T.	0
Little Club (Wash. 500)	4237	......	......	......	......	3	30	......	......	0	0	150	0
Little Club	4219	160	4	......	......	10	150	......	......	T.	0	10	0
Mayview	5874	......	......	......	......	0	0	......	......	0	0	T.	0
Salt Lake Club	3018	233	110	......	......	95	360	......	......	T.	0	10	0

POULARD WHEAT

Titanic	5535	7	0	......	......	25	0	......	......	0	0	0	......

DURUM WHEATS

Acme	5284	......	......	767	0	......	......	......	......	......	......	......	......
Arnautka	4064	15	0	420	0	25	238	......	0	0	0	0	0
Arnautka (Speltz Mars) (Minn. 337)	6236	......	......	......	......	23	0	......	......	0	0	10	0
Do	6236	......	......	......	......	48	0	180	0	0	0	30	0
Gharnovka	1443	......	......	620	0	10	120	......	......	0	0	100	0
Iumillo	1736	7	0	603	0	83	50	120	T.	0	0	T.	0
Kubanka	2094	......	......	......	......	0	0	......	......	0	0	T.	0
Kubanka No. 8	4063	7	0	257	0	80	4	121	10	0	0	T.	0
Marouani	2235	......	......	750	0	T.	0	......	......	0	0	450	0
Mindum (Minn. 470)	5296	......	......	800	0	96	0	396	0	0	0	75	0
Pentad (D-5)	3322	......	......	......	......	......	......	......	......	0	0	60	0

Table IV.—*Data obtained on the susceptibility of varieties of wheat, emmer, spelt, and einkorn to infection by Puccinia glumarum tritici as determined by records taken when the plants, grown in the nursery, were developing the fifth leaf and when they were heading, in one or more of the three years from 1918 to 1920, inclusive, at the Oregon Agricultural Experiment Station, Corvallis, Oreg.*—Continued

Group and variety.	C. I. No.	Varietal susceptibility.											
		1917–1918				1918–1919				1919–1920			
		Fall sown.		Spring sown.		Fall sown.		Spring sown.		Fall sown.		Spring sown.	
		5-leaf stage.	Heading.	5-leaf stage.	Heading.	5-leaf stage.	Heading.	5-leaf stage.	Heading.	5-leaf stage.	Heading.	5-leaf stage.	Heading.
EMMER													
Brown Winter	2483	0	0	......	......	T.	0	......	......	0	0	760	......
Khapli	4013	......	......	460	0	0	0	......	......	0	0	40	0
Vernal (White Spring) (Minn. 1165)	3686	......	......	163	0	0	0	40	0	0	0	0	0
Vernal (White Spring)	1522	......	......	800	0	......	......	......	......	......	......	......	......
Do	1524	......	......	743	0	0	0	333	0	0	0	10	0
Do	4781	......	......	760	0	......	......	......	......	......	......	......	......
Do	4781	......	......	......	......	0	0	347	0	0	0	0	0
SPELT													
Red Winter	1772	0	0	......	......	0	0	......	......	0	0	0	......
POLISH WHEAT													
Polish	5524	......	......	350	0	0	0	......	......	0	0	0	0
EINKORN													
Einkorn	2433	......	......	0	0	0	0	......	......	0	0	0	0

ADDITIONAL FIELD DATA

In addition to the information tabulated above on susceptibility of varieties in the rust nursery at Corvallis, Oreg., during the three seasons, many valuable field notes were recorded at various times and places where rust infection had developed under natural conditions.

At the Sherman County Branch Station, at Moro, Oreg., where extensive sowings of wheat varieties are made each year, there was a severe outbreak of stripe rust in the spring of 1919. This afforded an excellent opportunity to obtain data on the comparative susceptibility of some of the leading varieties of wheat grown at that station in 1919. The following table shows the ratings of these varieties, using the system of grading explained for Tables III and IV. The notes, taken just before the plants headed, are recorded in Table V.

TABLE V.—*Data showing the susceptibility of commercial wheat varieties to infection by stripe rust at the Sherman County Branch Station, Moro, Oreg., in 1919*

Variety.	C. I. No.	Varietal susceptibility.
Turkey	1558	425
Do	1571	410
Do	1756	325
Do	2998	415
Turkey (Local)	4429	390
Kanred	5146	525
Argentine	1569	560
Crimean	1437	510
Alberta Red	2979	675
Beloglina	2239	710
Theiss	1561	105
Nebraska No. 28	5147	355
Kharkof	5549	250
Jones Fife	6177	855
Hybrid 128	4512	630
Goldcoin (Fortyfold)	4156	635

During the season of 1919 notes were taken also on the stripe rust occurring on a number of wheat varieties growing at Moscow in the plots of the agronomy department of the University of Idaho. The notes were taken on June 20, when the grain was mostly headed. Apparently the rust was just beginning to appear at that time and, as no opportunity presented itself to inspect this grain again, it is not known that the figures in Table VI represent the maximum rust infection which appeared on these plots during that season. At any rate, they give some indication of the most susceptible varieties.

TABLE VI.—*Data showing susceptibility of wheat varieties to stripe rust after heads had appeared, when grown in plats at the University of Idaho, Moscow, in 1919*

Variety.	C. I. No.	Varietal susceptibility.
Prohibition	4068	0
Challenge (Webbs Challenge White)	4683	0
Rink	5868	0
White Winter	4684	0
Defiance	4354	0
Pacific Bluestem	3019	0
Kinney	5189	0
Red Russian	4509	0
Fultz	3416	50
Marquis	3641	0
Dawson (Dawson Goldenchaff)	4480	Trace.
Early Arcadia	4220	275
Red Wave	3500	385
Poole	3488	440
Poole (Harvest King)	4894	410
Jones Fife	3452	415
Haynes Bluestem	2874	0
Sonora	4293	0
Baart (Early Baart)	1697	Trace.
Stoner	2980	0
Golden Cross	5180	0
Kharkof	1442	0
Preston	2958	0
Hybrid 63	4157	Trace.

During the summer of 1920 there was an outbreak of stripe rust in the wheat classification nursery at Corvallis, Oreg. Table VII shows the comparative susceptibility of some of the varieties as indicated by the rust infection which appeared on them after the plants were all headed.

TABLE VII.—*Data showing susceptibility of wheat varieties to stripe rust when grown in the wheat classification nursery at Corvallis, Oreg., in 1920*

COMMON WHEATS

Group and variety.	C. I. No.	Varietal susceptibility.
Hard red spring:		
Chul	2227	50
Early Red Fife	4932	0
Haynes Bluestem	2874	0
Humpback	3690	0
Huston	5208	0
Kinney	5189	0
Marquis	3641	0
Pioneer	4324	0
Prelude	4323	400
Preston	3328	0
Preston (Velvet Chaff)	3318	0
Hard red winter:		
Alton (Ghirka Winter)	1438	0
Beloglina	1667	0
Kanred	5146	0
Kharkof	1442	0
Turkey	1558	0

TABLE VII.—*Data showing susceptibility of wheat varieties to stripe rust when grown in the wheat classification nursery at Corvallis, Oreg., in 1920*—Continued

COMMON WHEATS—continued

Group and variety.	C. I. No.	Varietal susceptibility.
Soft red winter:		
Climax (Jones Climax)	6203	275
Currell	3326	70
Currell (Golden Chaff)	5578	60
Diamond Grit	3385	100
Fulcaster	3406	50
Fultz	3416	150
Gipsy (Defiance)	5305	0
Goldencross	5180	T.
Grandprize	4876	400
Harold	6005	600
Harvest Queen	5314	0
Harvest Queen (Red Cross)	4882	0
Illini Chief	5406	0
Jones Fife	4468	250
Jones Fife (Super)	5544	600
Jones Longberry	5339	0
Leap	4823	0
Lofthouse	3275	0
Mealy	3358	20
Nebraska No. 28	5147	100
New Columbia	5946	20
Poole	3488	900
Poole (Harvest King)	5680	570
Prosperity (American Bronze)	5380	125
Red Clawson (Early Red Clawson)	3393	550
Red May	5336	20
Red May (Early Harvest)	4334	20
Red May (Early Ripe)	3394	150
Red May (Michigan Amber)	1969	0
Red May (Enterprise)	4854	T.
Red Russian	4509	0
Red Wave	3500	250
Rural New Yorker No. 6	5921	0
Rural New Yorker No. 57	3516	50
Squarehead	5234	0
Stoner	2980	0
Triplet	5408	0
Zimmerman	2907	0
White:		
Bearded Winter Fife	4204	100
Bobs	4990	T
Challenge (Webbs Challenge White)	4683	0
Dawson Goldenchaff	3342	50
Defiance	4347	5
Dicklow	3663	0
Early Arcadia	3390	125
Eaton	5682	0
Foisy	5242	0
Genesee Giant	1744	300
Goldcoin	2996	50
Gypsum (Colorado Special)	4762	0
Hard Federation	4980	0
Jumbuck	4608	0
Kofod	4337	0
New Zealand	6011	5
Oatka Chief	3481	300

TABLE VII.—*Data showing susceptibility of wheat varieties to stripe rust when grown in the wheat classification nursery at Corvallis, Oreg., in 1920*—Continued

COMMON WHEATS—continued

Group and variety.	C. I. No.	Varietal susceptibility.
White—Continued.		
Pacific Bluestem	4067	5
Prohibition	4068	0
Rink	5868	0
Satisfaction	5938	125
Satisfaction (Smith's Rustproof)	3588	25
Seneca Chief	3575	90
Silvercoin	6013	100
Sonora	3036	0
Sunset	6253	200
Surprise (White Russian)	5277	0
Talimka	2495	300
White Australian	3019	0
White Federation	4981	600
White Winter	5219	0
CLUB WHEATS		
Big Club	4257	0
Bluechaff	5256	100
Brown Glory	4240	200
Coppei	3088	150
Dale (Dale Gloria)	4155	950
Hybrid 60	5024	0
Hybrid 108	5025	300
Hybrid 123	4511	325
Hybrid 128	4512	200
Jenkin	5177	450
Little Club	4066	200
Mayview	5874	0
Redchaff	4241	150
MISCELLANEOUS WHEATS		
Alaska (poulard)	5988	0
Arnautka (durum)	1494	0
Einkorn	2433	0
Khapli emmer	4013	0
Kubanka (durum)	1440	0
Mindum (durum)	5296	0
Red Winter spelt	1772	0
Vernal (White Spring) emmer	1524	0
White Spring spelt	2968	0

GREENHOUSE EXPERIMENTS

The greenhouse studies on susceptibility of grain varieties to stripe rust were carried out in a wing of the greenhouse at the Oregon Agricultural Experiment Station, at Corvallis. The methods used were the same as previously outlined in the investigation of specialized races.

The results of the inoculations made in the greenhouse during the three seasons, 1917–18, 1918–19, and 1919–20, using 92 different varieties and strains of wheat, are embodied in Table VIII. The method used in computing data has already been explained in connection with Tables III and IV.

TABLE VIII.—*Data on susceptibility of varieties of wheat, emmer, spelt, and einkorn to stripe rust, including the number of separate experiments made each season and in all seasons, the number of plants inoculated and the number infected in each season and in all three seasons, and the annual and average degree of infection, when the plants were grown in the greenhouse at Corvallis, Oreg., in the three seasons, 1917–18, 1918–19, and 1919–20*

COMMON WHEATS

Group and variety.	C. I. No.	1917–18				1918–19				1919–20				Totals.			
		Number of—			Character of infection.	Number of—			Character of infection.	Number of—			Character of infection.	Number of—			Character of infection.
		Experiments.	Plants inoculated.	Plants infected.		Experiments.	Plants inoculated.	Plants infected.		Experiments.	Plants inoculated.	Plants infected.		Experiments.	Plants inoculated.	Plants infected.	
Hard red spring:																	
Barletta (Minn. 1148)	3297	......	......	......	......	3	36	4	0	......	......	......	......	3	36	4	0
Cedar	4117	......	......	......	......	1	15	13	2	4	43	21	3	5	58	34	2–3
Chul	2227–1	......	......	......	......	......	......	......	......	2	20	11	4	2	20	11	4
Chul (Yantagbay)	2404	......	......	......	......	......	......	......	......	2	20	4	2–3	2	20	4	2–3
Chul	2406	1	12	10	4	9	187	98	4	26	240	152	4	36	439	260	4
Dakota No. 5	......	1	15	0	......	......	......	......	......	......	......	......	......	1	15	0	0
Dakota No. 7	......	1	20	0	......	......	......	......	......	......	......	......	......	1	20	0	0
Ghirka (Ghirka Spring)	1517	......	......	......	......	1	13	2	1	2	20	10	1	3	33	12	1
Haynes Bluestem	2874	......	......	......	......	1	24	1	1	2	19	4	2	3	43	5	1–2
Kinney	5197	......	......	......	......	1	10	0	0	......	......	......	......	1	10	0	0
Do	5197	......	......	......	......	......	......	......	......	1	10	0	0	1	10	0	0
Koola	2203	1	15	10	4	2	29	21	3–4	......	......	......	......	3	44	31	3–4
Marquis	3641	......	......	......	......	......	......	......	......	3	33	14	4	3	33	14	4
Pioneer	4234	......	......	......	......	1	14	0	0	......	......	......	......	1	14	0	0
Preston	3081	......	......	......	......	1	20	0	0	3	30	0	0	4	50	0	0
Red Fife	4932	......	......	......	......	1	14	0	0	2	20	0	0	3	34	0	0
Hard red winter:																	
Kanred	5146	2	33	33	4	9	158	47	4	3	50	17	3–4	14	241	97	3–4
Kharkof	2193	......	......	......	......	1	20	10	2	2	20	2	3	3	40	12	2–3

P—1066	5879	...	...	...	...	...	...	...	...	2	20	2	3	2	20	2	3
P—1068	5880	...	...	...	...	...	...	...	...	2	25	6	3	2	25	6	3
Turkey	1558	1	16	0	0	5	46	12	4	3	36	10	4	9	98	22	4
Soft red winter:																	
Banner Berkeley	...	...	...	...	...	1	36	11	4	...	...	...	...	1	36	11	4
Fulcaster	3406	...	...	...	...	2	44	14	3–4	...	...	...	...	2	44	14	3–4
Fultz	3416	...	...	...	...	1	19	0	0	...	...	...	...	1	19	0	0
Goldencross	4564	...	...	...	...	1	22	0	0	3	30	0	0	4	52	0	0
Grandprize	4876	...	...	...	...	1	12	8	4	2	20	14	4	3	32	22	4
Harvest Queen	5314	...	...	...	...	...	...	...	...	2	20	0	0	2	20	0	0
Illini Chief	5406	...	...	...	...	1	26	0	0	2	20	0	0	3	46	0	0
Jones Fife	3452	...	...	...	...	...	...	...	...	2	30	2	4	2	30	2	4
Do	4468	1	18	15	4	4	70	34	4	4	61	9	4	9	149	58	4
Do	4468	...	...	...	...	...	...	...	...	2	20	2	1–2	2	20	2	1–2
Do	5608	...	...	...	...	...	...	...	...	3	30	7	4	3	30	7	4
Nebraska No. 28	5147	...	...	...	...	1	17	9	4	...	...	...	...	1	17	9	4
Purdue No. 1	4871	...	...	...	...	...	...	...	...	3	33	20	2–4	3	33	20	2–4
Red Russian	4509	...	...	...	...	1	12	0	0	1	10	0	0	2	22	0	0
Red Russian (Wash. 270).	4509	...	...	...	...	...	...	...	...	1	10	0	0	1	10	0	0
Squarehead	5234	...	...	...	...	1	29	0	0	2	20	0	0	3	49	0	0
Stoner	2980	...	...	...	...	1	17	11	4	2	30	5	4	3	47	16	4
Zimmerman	2907	...	...	...	...	1	17	0	0	...	...	...	...	1	17	0	0
White:																	
Arcadian (Early Arcadia).	4220	...	...	...	...	...	...	...	...	3	27	9	4	3	27	9	4
Baart (Early Baart)	1697	2	35	32	4	5	82	62	4	6	69	23	4	13	186	117	4
Bobs	2826	...	...	...	...	1	27	15	3	3	20	3	2	4	47	18	2–3
Dicklow	4758	...	...	...	...	1	23	0	0	4	40	0	5	5	63	0	0
Eaton	4682	...	...	...	...	1	13	0	0	2	20	0	0	3	33	0	0
Federation	4734	...	...	...	...	1	18	10	4	2	20	6	3	3	38	16	3–4
Florence	4170	...	...	...	...	2	22	8	0	...	...	...	...	2	22	8	0
Foisy	5253	...	...	...	...	...	...	...	...	2	20	1	2	2	20	1	2
Goldcoin (American Banner).	3016	...	...	...	...	2	40	36	3–4	5	36	19	4	7	96	55	3–4
Goldcoin (Fortyfold)	4156	...	...	...	...	...	...	...	...	4	41	8	3	4	41	8	3

TABLE VIII.—*Data on susceptibility of varieties of wheat, emmer, spelt, and einkorn to stripe rust, including the number of separate experiments made each season and in all seasons, the number of plants inoculated and the number infected in each season and in all three seasons, and the annual and average degree of infection, when the plants were grown in the greenhouse at Corvallis, Oreg., in the three seasons, 1917–18, 1918–19, and 1919–20*—Continued

COMMON WHEATS—continued

Group and variety.	C. I. No.	1917–18				1918–19				1919–20				Totals.			
		Number of—			Character of infection.	Number of—			Character of infection.	Number of—			Character of infection.	Number of—			Character of infection.
		Experiments.	Plants inoculated.	Plants infected.		Experiments.	Plants inoculated.	Plants infected.		Experiments.	Plants inoculated.	Plants infected.		Experiments.	Plants inoculated.	Plants infected.	
White—Continued.																	
Pacific Blu st	5231	.....	.....	.....	.....	1	17	2	2	2	20	1	2	3	37	3	2
Rink	5865	.....	.....	.....	.....	.....	.....	.....	.....	5	50	33	2	5	50	33	2
Royalton (Minn. 1037)	4968	1	8	1	1	.....	.....	.....	.....	.....	.....	.....	.....	1	8	1	1
Sonora	3036	1	68	4	1	6	42	11	1	3	30	2	1	10	140	17	1
Do	4293	.....	.....	.....	.....	1	18	0	0	2	17	0	0	2	35	0	0
Do	4501	.....	.....	.....	.....	.....	.....	.....	.....	2	20	0	0	2	20	0	0
Talimka	2495	1	35	27	4	5	79	62	4	35	983	451	4	41	1,097	540	4
White Winter	4684	.....	.....	.....	.....	.....	.....	.....	.....	1	10	0	0	1	10	0	0
Do	5232	.....	.....	.....	.....	.....	.....	.....	.....	2	23	0	0	2	23	0	0
Wilhelmina	4193	.....	.....	.....	.....	1	13	0	0	3	34	0	0	4	47	0	0
Miscellaneous:																	
Black Persian	2442	1	10	4	3	.....	.....	.....	.....	.....	.....	.....	.....	1	10	4	3
Chul x Turkey	.....	.....	.....	.....	.....	2	55	40	4	.....	.....	.....	.....	2	55	40	4
Hansia Broach	4690	.....	.....	.....	.....	.....	.....	.....	.....	3	39	22	4	3	39	22	4
Popatia Nadiad	4696	1	14	14	4	7	76	35	4	1	11	2	4	9	101	51	4
Western Sweepstakes	5607	.....	.....	.....	.....	1	23	21	4	2	20	2	3	3	43	23	3–4

CLUB WHEATS

Dale (Dale Gloria)	4231	1	27	23	4	2	46	25	4	3	27	5	3	6	100	53	3–4
Hybrid 128	4512					1	17	13	4					1	17	13	4
Hybrid 143	4160									2	20	1	2	2	20	1	2
Little Club	4219	1	38	26	4	2	24	9	4	4	53	8	4	7	115	43	4
Salt Lake Club	3018					1	25	22	4					1	25	22	4

POULARD WHEAT

Titanic	5535					1	13	5	0	2	20	4	2	3	33	9	0–2

DURUM WHEATS

Arnautka	4064					1	16	15	4	1	10	0	0	2	26	15	4
Arnautka (Speltz Mars)	6236					1	4	2	4					1	4	2	4
Beloturka	1513									2	20	4	2	2	20	4	2
Black Don	2100	1	15	12	4									1	15	12	4
Iumillo	1736	1	13	8	2	1	26	4	2	3	30	10	1	5	69	22	1–2
Kubanka No. 8	4063	2	41	39	4	1	22	6	4					3	63	45	4
Marouani	2235	1	15	13	4									1	15	13	4

EMMER

Black Winter	2337	2	46	42	4					2	19	9	4	4	65	51	4
Brown Winter	2483	2	43	40	4	2	14	7	4					4	57	47	4
Khapli	4013	1	11	0	0									1	11	0	0
Vernal	1522	1	16	15	2									1	16	15	2
Do	1524	1	15	10	2									1	15	10	2
Do	1526	1	35	35	2									1	35	35	2
Do	4781	1	30	24	1									1	30	24	1
Vernal (Minn. 1165)	3686	3	69	33	0–1									3	69	33	0–1

TABLE VIII.—*Data on susceptibility of varieties of wheat, emmer, spelt, and einkorn to stripe rust, including the number of separate experiments made each season and in all seasons, the number of plants inoculated and the number infected in each season and in all three seasons, and the annual and average degree of infection, when the plants were grown in the greenhouse at Corvallis, Oreg., in the three seasons, 1917–18, 1918–19, and 1919–20*—Continued

SPELT

Group and variety.	C. I. No.	1917–18 Number of— Experiments.	1917–18 Number of— Plants inoculated.	1917–18 Number of— Plants infected.	1917–18 Character of infection.	1918–19 Number of— Experiments.	1918–19 Number of— Plants inoculated.	1918–19 Number of— Plants infected.	1918–19 Character of infection.	1919–20 Number of— Experiments.	1919–20 Number of— Plants inoculated.	1919–20 Number of— Plants infected.	1919–20 Character of infection.	Totals. Number of— Experiments.	Totals. Number of— Plants inoculated.	Totals. Number of— Plants infected.	Totals. Character of infection.
Red Winter	1772	1	30	1	2	......	......	......	......	......	......	......	......	1	30	1	2
EINKORN																	
Einkorn	2433	1	20	0	0	......	......	......	......	......	......	......	......	1	20	0	0
Do	2973	1	24	20	1	......	......	......	......	......	......	......	......	1	24	20	1

NURSERY AND GREENHOUSE RESULTS COMPARED

Table IX presents a comparison of the results obtained in both the nursery and greenhouse with certain varieties which have proved very resistant in all these trials. No varieties are listed unless they were grown three seasons in the nursery and tested for resistance there.

TABLE IX.—*Data showing summarized results of nursery and greenhouse experiments on susceptibility of wheat varieties to infection by P. glumarum tritici*

Variety.	C. I. No.	Nursery.		Greenhouse.	
		Number of experiments.	Average susceptibility.	Number of experiments.	Character of infection.
Barletta	3297	4	0.5	3	0
Dicklow	4758	8	12	5	0
Foisy	5253	6	17	2	2
Einkorn	2433	4	0	1	0
Fultz	3416	5	14	1	0
Harvest Queen	5314	4	1	2	0
Haynes Bluestem	2874	6	33	3	1–2
Kharkof	2193	5	10	3	2–3
Kinney	5197	6	Trace.	1	0
Preston	3081	10	15	4	0
Wilhelmina	4193	6	6	4	0
Early Red Fife	4932	6	22	3	0
Red Russian	4509	6	4	2	0
Red Winter spelt	1772	5	0	1	1
Rink	5868	6	Trace.	5	2
Royalton	4968	6	0	1	1
Sonora	3036	6	4	10	1
Challenge (Webbs Challenge White)	4683	6	Trace.	....	....
White Winter	4684	6	Trace.	1	0

As shown by Tables III to IX, there has been a marked difference in susceptibility to stripe rust in the different varieties of wheat. Further study doubtless will show whether there are strains of the rust which are able to attack some of these varieties which have proved resistant to the strains which have been under observation. The difference in susceptibility to stripe rust in the various varieties of wheat which have been studied seems to be much more marked than in the case of either stem rust or leaf rust.

Various European workers have referred to this very marked difference in susceptibility to *P. glumarum* as it appears in Europe. Henning (*8*) states in this connection, "The economic importance of the yellow rust has recently, in our country (Sweden), been considered rather inferior, since we, in the latter part of the nineteenth century, have demonstrated that the different varieties of wheat show a very unlike susceptibility to this disease; moreover, we have succeeded in producing varieties, by means of crossing, which possess a marked power of resistance toward yellow rust. However, we have learned recently that this power of resistance is quite variable, so that we must give some further attention to this kind of rust."

Comparatively few of the different varieties studied showed infection in the heads. This is the type of infection which is the most destructive, as the yield may be very materially reduced when the heads become infected. A discussion of this type of injury was given in one of the earlier papers of this series (*10*). It also is noteworthy that few of the wheat varieties which are commonly grown in the western portion of the United States where stripe rust is found are very susceptible to the rust. Exceptions to this are several of the club varieties, Early Baart, Jones Fife, and a few others.

A study of the foregoing results also will show that the varieties which have proved more or less resistant to leaf rust and stem rust have not shown, in every case, a similar resistance to stripe rust. Notable examples of this are several of the durum wheats which have been shown to be resistant to most strains of *Puccinia graminis tritici*. Khapli (C. I. 4013), an emmer which Hayes, Parker, and Kurtzweil (*6*) found resistant to all biologic forms of stem rust thus far isolated, is not especially resistant to the strains of stripe rust with which it has been inoculated. Jenkin and Sampson (*11*) state that comparatively few of the wheat varieties which they tested were resistant to both black rust (Puccinia graminis) and yellow rust (Puccinia glumarum).

Hiltner (*9, p. 83*) reports that in Germany spring wheat suffers more than winter wheat from the attack of *P. glumarum*. He also states that the club varieties as a class appear to be especially susceptible and that those varieties with broad leaves were not so often attacked by the rust. Von Kirchner (*12*) gives 20.4 per cent as the average stripe-rust infection on winter wheat for a number of years, while spring wheat for the same number of years showed only 16.2 per cent infection. Jenkin and Sampson (*11*) state that autumn varieties were found to be more susceptible to black rust and the spring varieties to yellow rust. Schneiderhan, in the unpublished report of the Sherman County Branch Station, Moro, Oreg., for 1916, reports that when certain varieties were grown as both spring and winter wheats, those sown in the fall developed the larger percentages of infection of stripe rust.

Examination of Tables III and IV shows that leaf infection in the seedling stage usually was much greater in spring-sown wheat. Infection at heading time, on the contrary, was in most cases much greater in winter wheat. It seems reasonable to suppose that both seasonal and regional differences in climate will determine to a certain extent whether fall-sown or spring-sown wheat becomes more heavily infected with stripe rust.

Histological studies of resistant and susceptible varieties of wheat attacked by stripe rust have not been made. The general external macroscopic evidences of resistance appear to be similar to those described by Stakman (*17*) for *Puccinia graminis tritici* E. and H. and by Parker (*15*) for *Puccinia graminis avenae* E. and H. Plates 3 to 5 illustrate the typical effect of *P. glumarum tritici* upon resistant hosts. Large areas of killed tissue develop on the portions of the leaves inoculated, followed by the production of very few if any uredinia. Upon varieties which are moderately resistant, urediniospores often are produced in abundance but no spread of the rust occurs from the parts of the leaf inoculated, and these portions are soon killed.

Parker (*15*) interprets purple blotches adjacent to the uredinia as evidence of resistance of oats to *P. graminis avenae.* As indicated in Table II and as illustrated in Plate 3, A, dark brown spots often develop on certain grass hosts when inoculated with urediniospores of *P. glumarum tritici.* Similar brown discoloration often appears around the edge of the uredinia on fairly susceptible grasses such as *Bromus sterilis*, *Bromus sitchensis*, and others. This type of reaction has been noted only on certain species of grasses. Parker (*15*) also found that the production of telia of crown rust on seedlings of oats in the greenhouse was an indication of resistance. Although in a few cases telia have developed in the greenhouse upon wheat seedlings in connection with the work herein reported, this has been interpreted as being due to the effect of certain environmental conditions upon the host rather than as a sign of resistance.

SUMMARY

Field collections of *Puccinia glumarum* in the western part of the United States have been made on wheat, barley, rye, spelt, and emmer, as well as on 33 wild grasses.

It has been shown by artificial inoculation that the rust also will infect 26 additional grass hosts. This makes a total of 59 species of wild grasses which are hosts for this rust as it occurs in the United States.

The common specialized variety of stripe rust in the United States is the one peculiar to wheat, *P. glumarum tritici* Erikss. and Henn. Field observations indicate that the variety developing on barley, *P. glumarum hordei* E. &. H., also occurs in this country.

The specialized variety from wheat also will infect rye moderately and barley slightly. Inoculation experiments have shown that this variety also will infect 47 wild grasses. This list includes 19 species of Bromus; 11 species of Agropyron; 7 species of Hordeum; 7 species of Elymus, and one species each of Hystrix, Phalaris, and Sitanion. Stripe rust has been collected in the field on twelve additional grass hosts, but it has not yet been possible to ascertain if these also are hosts for the specialized variety from wheat.

There has been some evidence that there are several strains of the different grass species which react differently to the same variety of the rust. There has been some indication also that there are two or more strains or specialized forms of *P. glumarum tritici.*

Varieties of wheat and wheat allies to the number of 163 have been tested for resistance to stripe rust in a rust nursery at Corvallis, Oreg., where an epiphytotic of this rust was produced artificially each year for three years. All of these varieties were grown for two years and part of them for three years. The results of these experiments are presented in tabular form.

Ninety-two varieties of wheat have been tested for resistance to stripe rust in the greenhouse. Some of these were studied three years, some of them in two years, and some of them in only one year. The results of these experiments are given in tabular form. With few exceptions the results of the field and greenhouse studies have agreed quite closely.

There is a very marked difference in the susceptibility of various wheat varieties to stripe rust. More of the common wheat varieties appear to be resistant to stripe rust than are resistant to stem rust. When more strains of stripe rust are tried this may not continue to be the case. Comparatively few of these varieties which were studied became infected

in the head under the conditions of the experiment. Varieties which developed head infection were greatly reduced in yield.

Leaf infection in the seedling stage has been more severe in spring-sown wheat. Leaf infection at heading time has been more severe in fall-sown wheat.

Several varieties have proved very resistant to the strains of the rust studied. Resistance is evidenced by the development of large areas of killed tissue in the portions of the leaves inoculated, followed by the production of few or no uredinia.

Dark brown blotches and browning around the edges of inoculated areas have developed upon certain grass hosts. This characteristic appears to be specific for certain grasses and has not been considered a general sign of resistance.

Telia have been formed in the greenhouse in a few cases under certain conditions. This was thought to be due to the conditions surrounding the host and was not regarded as a sign of resistance.

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PLATE 1

Method of growing the wheat seedlings for study of specialized races.

A.—Bell jar and battery jar used as incubation chambers, and pots containing wheat seedlings, showing method of separating the plants in each pot into two groups, one group being inoculated and the other used as control.

B.—Tub covered with window sash, used as an incubation chamber. Half the plants in the pots were inoculated, the pot placed in the tub in about 2 inches of water and the tub covered with the sash for 48 hours.


C

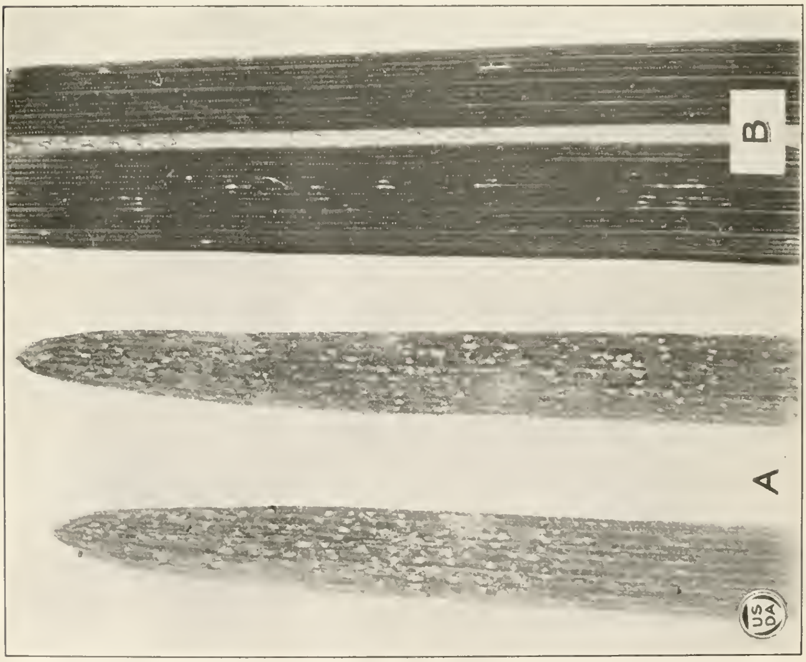
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PLATE 2

Normal development of uredinia of *Puccinia glumarum tritici* on wheat leaves.

A.—Distal portion of a leaf of a seedling of Chul, showing abundant infection and production of uredinia.

B.—Portion of an older leaf of Chul, showing very abundant infection in the form of a single, longitudinal stripe.

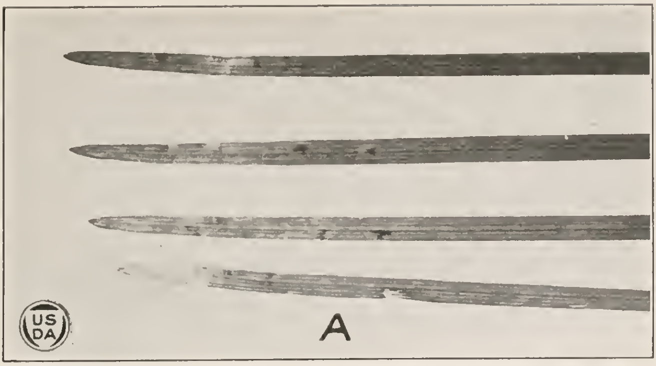
C.—Portion of a leaf of Little Club wheat, showing abundant infection and production of uredinia.

PLATE 3

Portions of inoculated leaves showing flecks or areas of tissue injured or destroyed by the fungus but no permanent infection resulting. This condition represents grade o in the five gradations of susceptibility to infection by *Puccinia glumarum tritici*.

A.—Portions of mature leaves of *Bromus carinatus* showing distinct brown blotches.

B.—Portions of mature leaves of Red Russian wheat showing large spots of killed tissue.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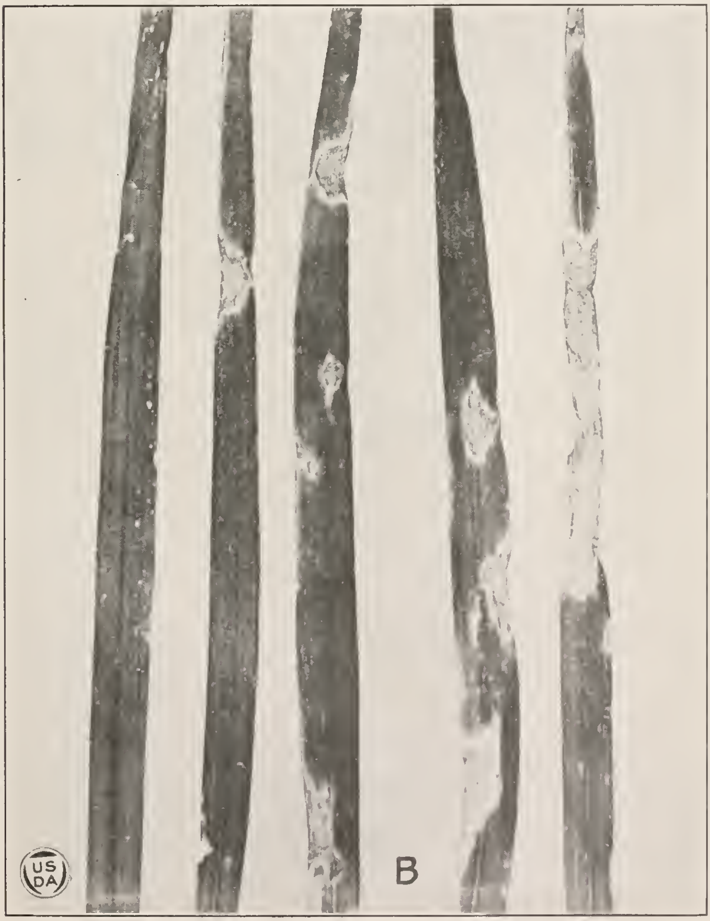
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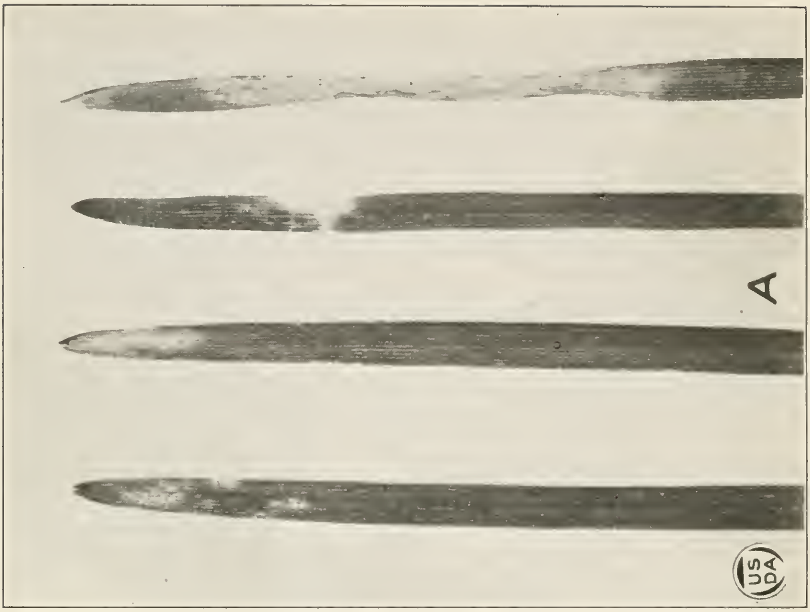

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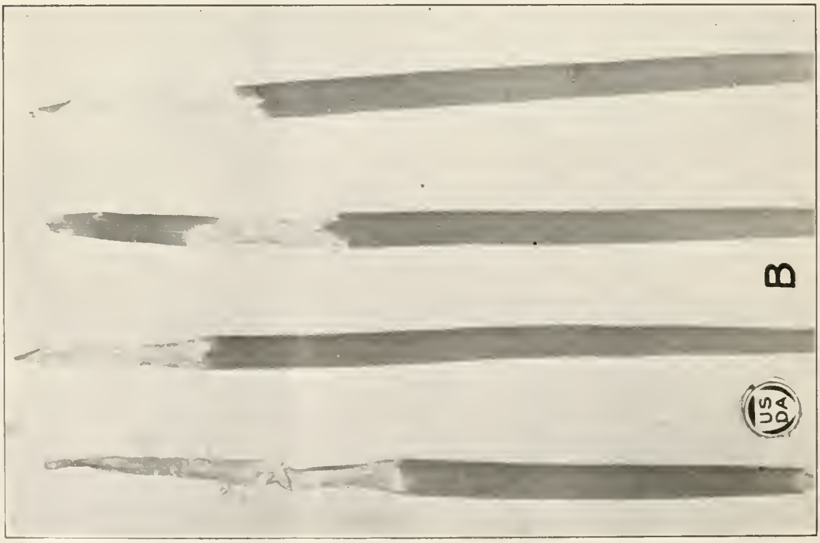

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PLATE 4

Portions of inoculated leaves showing flecks or areas of tissue injured or destroyed by the fungus but no permanent infection resulting. This condition represents grade 0 in the five gradations of susceptibility to infection by *Puccinia glumarum tritici.*

A.—Distal portion of leaves of Barletta wheat (C. I. No. 3297).

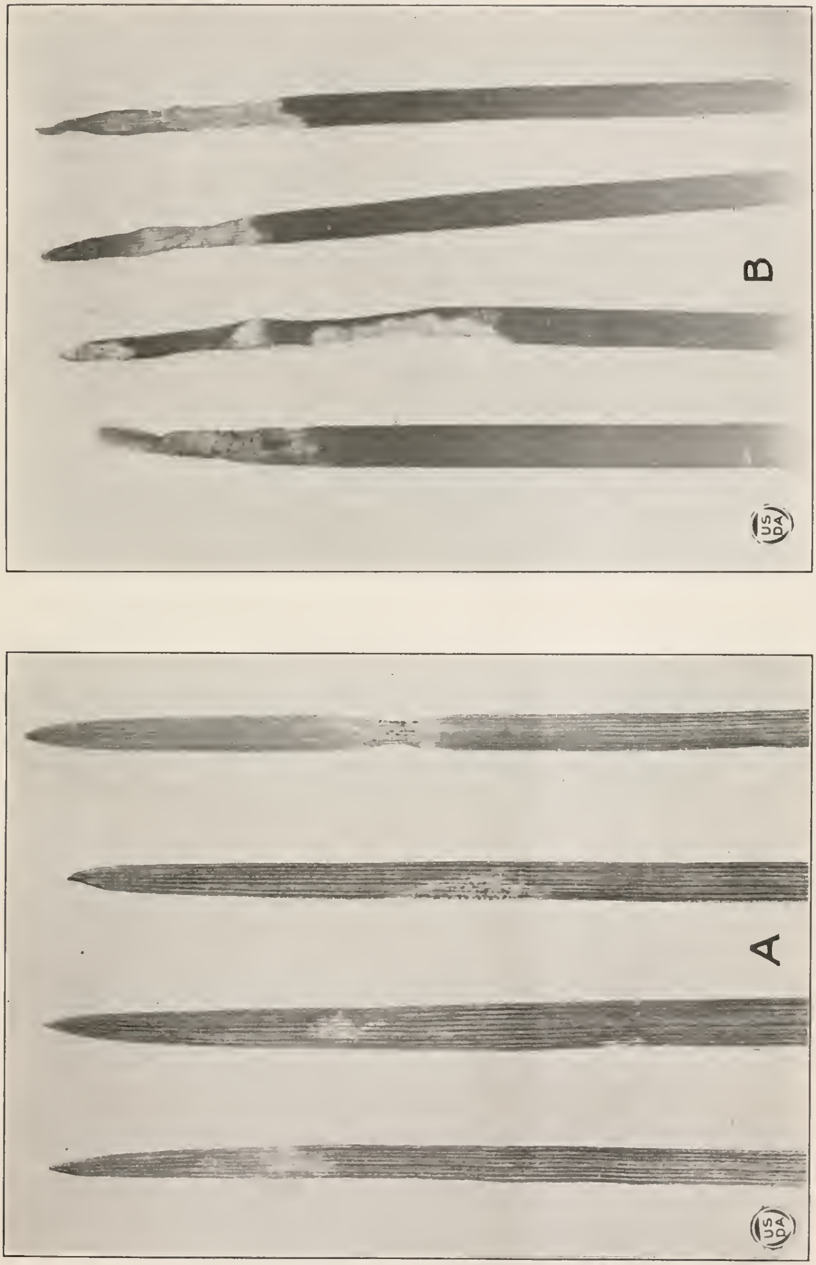
B.—Distal portion of leaves of Einkorn (C. I. No. 2433).

PLATE 5

Portions of inoculated leaves showing uredinia few or minute, usually surrounded by areas of dead or discolored tissue. This condition represents grade 1 in the five gradations of susceptibility to infection by stripe rust.

A.—Distal portions of leaves of Sonora wheat (C. I. No. 3036).

B.—Distal portions of leaves of Einkorn (C. I. No. 2973) 18 days after inoculation.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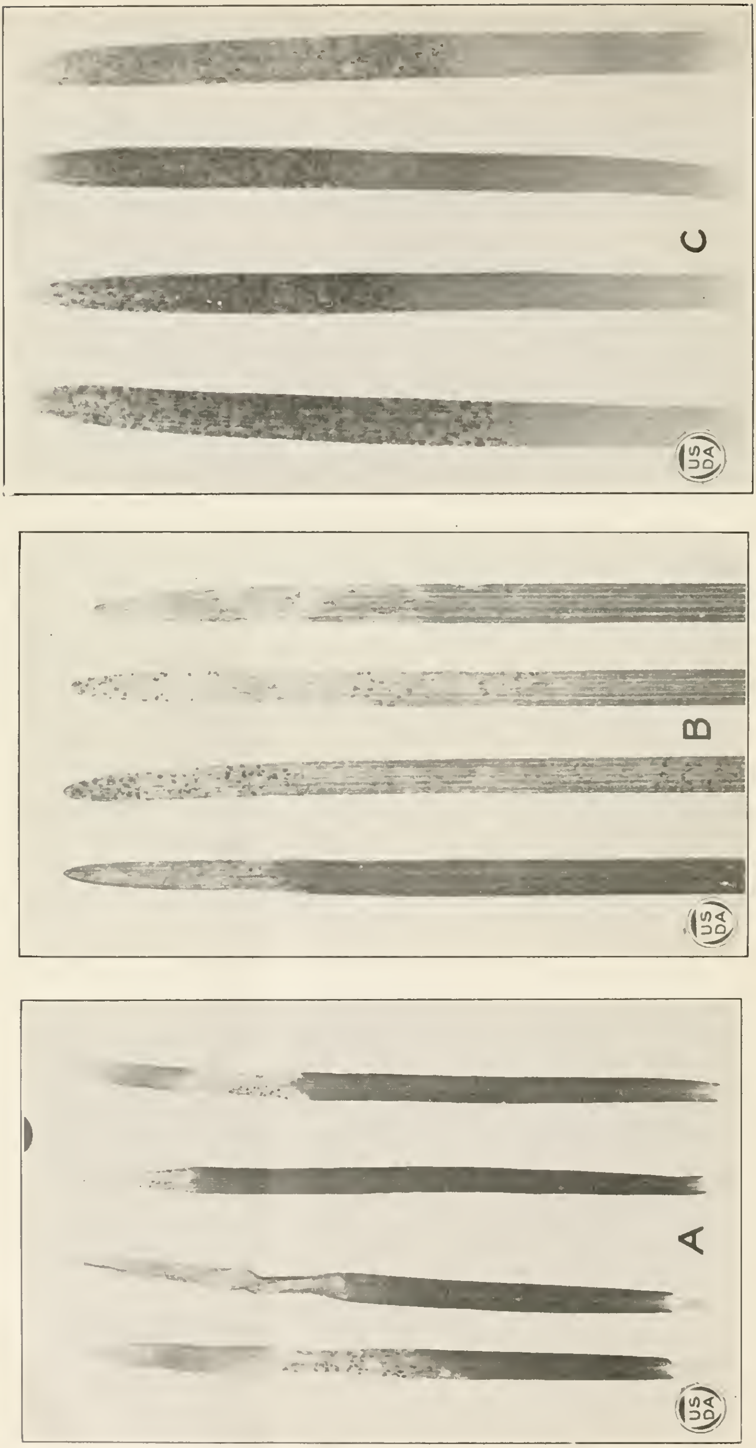
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B
A

4026

PLATE 6

Portions of inoculated leaves showing effects of inoculation with *Puccinia glumarum tritici*.

A.—Distal portions of leaves of Red Winter spelt (C. I. No. 1772), showing normal uredinia, but few and scattered, with leaf tissue abundantly discolored. This condition represents grade 2 of the five gradations in susceptibility to infection by stripe rust.

B.—Distal portions of leaves of *Bromus sterilis*, showing uredinia normal and moderately abundant, with but little discoloration of leaf tissue. This condition represents grade 3 of the five gradations in susceptibility to infection by stripe rust.

C.—Distal portion of leaves of Black Winter emmer, showing uredinia normal and abundant, scattered uniformly over surface of leaf, with no discoloration in the early stages of infection. This condition represents a grade of 4, or very susceptible, in the five gradations in susceptibility to infection by stripe rust.

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ORIGIN AND CONTROL OF APPLE-BLOTCH CANKERS[1]

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INTRODUCTION

On account of the great significance of the twig cankers as a means of overwintering and source of early infection of the apple-blotch fungus (*Phyllosticta solitaria* E. and E.), knowledge of the mode of origin of these cankers is highly important. Likewise any new light on methods of canker prevention and eradication is much to be desired.

The identity and significance of the blotch cankers were discovered about the same time by Scott and Rorer (*14*)[3] and by Sheldon (*16*), and petiole lesions were described by Sheldon and later by Scott and Rorer (*15*), who made an incidental observation of peculiar interest. They found a large percentage of the fruit buds in an orchard of Limbertwig, Missouri, and Ben Davis trees in Arkansas being killed in midsummer and attributed this in part to the apple-blotch fungus (*15, p. 11*) which according to their observations and cultural work "extended down from diseased leaf petioles into the twigs at the base of the buds, which were soon killed." Apparently, no significance was attached to this phenomenon in connection with the origin of cankers, since they mention spore infection of the twigs.

Lewis (*10, p. 528, 533*), who studied this disease on the Missouri variety in Kansas, states in connection with fruit spur cankers that "the fungus sometimes enters from the leaf stem, and at other times through the new growth just below the bud," and in connection with the importance of leaf infection he mentions the "possible infection of the twig from the petiole."

Roberts (*12, 13*) was able to produce cankers on young twigs and watersprouts by spraying with a water suspension of the spores, but was unable to infect older branches in this way and also was unable to cause infection of twigs by wound inoculation. These results would indicate that cankers are the result of germ tube infection through the uninjured epidermis of very young wood.

A study of the blotch cankers on the Northwestern variety at Mooresville (orchard of Mr. D. B. Johnson) and Knightstown (orchard of Mr. J. B. Hamer), in central Indiana, from 1919 to 1922, and on the Oldenburg variety at Mitchell, in southern Indiana, in 1921 and 1922, indicates that a large percentage of the cankers on twigs are the result of invasion from infected petioles rather than of direct spore infection (7). The

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[3] Reference is made by number (italic) to "Literature cited," p. 417-418.

evidence and the obvious deductions are presented herein together with an account of the spray control of canker formation and the eradication of cankers in young orchards.

LOCATION OF CANKERS

One of the outstanding features of the blotch disease in Indiana is that a very high percentage of the cankers on the bearing wood are located at leaf scars (Pl. 1, C, D, F). This condition is very evident on wood not over 3 or 4 years old, but even the older cankers are generally located at leaf nodes and crotches or about the bases of spurs that have developed from dormant buds (Pl. 3, A).

In September, 1921, records taken on 632 cankers on 1920 wood of the lower limbs of Northwestern trees showed that 624, or 98 per cent, were located at leaf scars, and in the fall of 1922 records taken on 967 cankers on 1921 wood of the same trees showed that 963, or 99.5 per cent, were located at leaf scars. Observations made in October, 1921, on 187 Oldenburg twigs of the current season showed that out of 129 small cankers which had already appeared 61 per cent were located at leaf scars, 16 per cent at the bases of terminal bud scales, and 23 per cent between the scars. Observations made in July, 1922, on the 1921 wood of 80 Oldenburg twigs showed that out of 553 cankers noted 87 per cent were at leaf scars, 5 per cent at the bases of terminal bud scales (Pl. 1, A), and 8 per cent between leaf scars.

On suckers, watersprouts, seedlings, and nursery stock a considerable proportion of the cankers occur between the leaf scars (Pl. 1, B). Counts made on watersprouts showed that 44 out of 50 bore cankers between the leaf scars on growth of the current season. Counts made on suckers of the current season showed that out of 92 lesions noted, 58 per cent were between the scars. On the first-year scion wood of 10 nursery trees 159 blotch cankers were counted, and 58 per cent of these occurred between the leaf scars and the remainder at the scars (Pl. 1, E). It would seem, therefore, that on rapidly growing wood cankers may occur rather generally between the leaf scars.

With respect to location there are four types of canker, (1) at the leaf scars, (2) at the bases of terminal bud scales, (3) at the bases of spurs, and (4) between the leaf scars. Of these the first is the prevalent type on bearing wood and consequently the most important.

DATE OF APPEARANCE OF CANKERS

Since Roberts (*12*) found only young twigs susceptible to spore infection, and since the infection period is early in the season, cankers resulting from direct spore infection should become visible during the first season. This has proved to be the case. The cankers between the leaf scars may be seen the first season and these undoubtedly result from direct spore infection. But in central Indiana the cankers at the leaf scars, which are the prevalent type on bearing wood, do not as a rule become visible until the following spring. It should be noted, however, that in southern Indiana, a larger percentage appear the first season than in the central part of the State.

In the fall of 1920, 35 twigs of 1920 wood on a badly cankered Northwestern tree at Mooresville were tagged and carefully examined. Three small cankers at leaf scars and three cankers between leaf scars were visible. No more of the latter type were evident. On March 22, 1921,

6 leaf-scar cankers were visible, on April 27 there were 68, by May 20, 95 had appeared, and by September 15, 109 were present on these twigs. Thus the three cankers between leaf scars appeared in the fall of the first season, and 103 out of 109, or 95 per cent, of the leaf-scar cankers did not appear until the second season. Fifty-seven per cent appeared between March 22 and April 27 and previous to the petal-fall period, 25 per cent between April 27 and May 20, and 12 per cent after May 20.

On September 27, 1921, eight twigs on a badly cankered Northwestern tree at Knightstown were tagged and carefully examined. Four cankers were found in the fall, and by April 4, 1922, 6 were visible. By April 25 only 1 more lesion had appeared, but between this date and May 25, 39 cankers became visible and only 3 more appeared later, making a total of 49 cankers, of which 39, or 79 per cent, appeared between April 25 and May 25. All but one, which appeared the first fall, were located at leaf scars.

It is of interest to note that the cankers appeared about a month later in 1922 than those studied at Mooresville in 1921. This difference may be attributed in part to the fact that the spring season at Mooresville is always more advanced than at Knightstown, which is only 45 miles distant and 12 miles farther north. In 1920 the petals fell nine days later at Knightstown than at Mooresville. A more important reason is, however, that the spring of 1922 was later than that of 1921. The petals fell six days later at Knightstown than in 1921. March, 1922, averaged 7.4° F. cooler than March, 1921, and April, 1.4° cooler, according to the records of the Federal Weather Bureau.

The appearance of the majority of these cankers early in the spring of the second season shows that infection must have occurred the previous season and that the mycelium must have been present in the cortex of the twig all winter.

Since the early blotch sprays, as will be shown later, prevent infection, it seems that all spore infection must occur during a rather definite period soon after petal-fall. Consequently, the delayed appearance and the variation in date of appearance of the leaf-scar cankers is difficult to explain upon the basis of direct spore infection. On the other hand, these phenomena are readily explained upon the basis of mycelial invasion of the twig from basal petiole lesions because the mycelium might cross from the petiole to the cortex of the twig at any time during the season. The problem here is quite unlike that found by Wiltshire (*17*) in England in connection with the late fall and early spring spore infection of leaf scars by the apple-canker fungus (*Nectria ditissima*) which acts somewhat as a wound parasite.

The cankers do not appear as gradually enlarging spots but are of considerable size when they first become visible, and the portion which thus makes its appearance is soon delimited by a fissure in the cortex (Pl. 1, C, D, F). The mycelium apparently penetrates a considerable area of bark tissue before its effects become visible.

PREVALENCE OF PETIOLE LESIONS

Petiole lesions (Pl. 1, G, H, I,) are very abundant on the lower limbs of cankered trees. Observations made on unsprayed Northwestern trees in 1920 showed that out of 4,937 leaves examined 1,633, or 33 per cent, bore petiole lesions. In 1921, 5,099 leaves on Northwestern trees were exam-

ined and 2,643 of these, or 52 per cent, bore petiole lesions, and in 1922 84 per cent of the 2,099 leaves examined bore petiole lesions. Out of 1,206 leaves on unsprayed Oldenburg trees examined in 1921, 93 per cent showed petiole lesions and, out of 893 leaves from Ben Davis trees, 88 per cent showed petiole infection. From these figures it may be seen that a very high percentage of the leaves on the lower limbs bear petiole lesions. In the majority of cases these lesions are located very near the base of the petiole (Pl. 1, G, H, I,), often in close proximity to the abscission layer.

Incidentally, it is of interest to note that pycnidia containing viable spores are commonly present in these petiole lesions on leaves that have lain over winter on the ground, but no signs of an ascigerous stage have been found.

VISIBLE TWIG INVASION FROM PETIOLE LESIONS

In some cases the lesion at the base of a petiole enlarges until it visibly crosses the abscission layer and invades the tissues of the twig (Pl. 1, I). On the 35 twigs tagged at Mooresville in 1920, there were 155 leaves with basal petiole lesions and in three cases the lesion extended across to the twig tissue. On the eight twigs tagged at Knightstown in the fall of 1921, 65 out of the 105 leaves bore basal petiole lesions and in three cases the lesion had crossed to the twig.

At Mitchell, in the fall of 1921, 129 lesions were noted on Oldenburg twigs of the current season, of which 79, or 61 per cent, were located at leaf scars, and although a large proportion of the leaves at these scars had fallen, 38 per cent of these leaf-scar lesions were obviously extensions from basal petiole lesions.

Thus, in many cases actual twig invasion from petiole lesions may be observed during the current season, particularly in southern Indiana, but as previously noted, the great majority of the twig lesions do not become visible until long after the leaf has fallen.

CORRELATION OF LEAF-SCAR CANKERS AND BASAL PETIOLE LESIONS

In order to determine whether or not cankers appear only at leaf scars to which infected petioles have been attached, 35 twigs on a Northwestern tree at Mooresville were tagged in the fall of 1920 and the location of each leaf with a basal petiole lesion was recorded. There were leaves with basal petiole lesions at 155 scars, and at 109, or 70 per cent, of these leaf scars, blotch cankers appeared, mostly during April and May of the following year, as previously noted. A total of 146 cankers developed on these twigs and 109, or 74 per cent, developed where an infected petiole had been attached. Thirty-two cankers, or 22 per cent, were located at scars where no basal petiole infection had been recorded; the origin of these cankers is not clearly understood. It is possible that small petiole lesions were overlooked.

To ascertain more accurately the correlation between petiole lesions and twig cankers, careful records were taken on the leaves on eight twigs of a Northwestern tree at Knightstown on September 27, 1921. There were 107 leaves on these twigs and 76 bore petiole lesions. In each case, the distance of the lower margin of the lowest lesion from the abscission layer was recorded in millimeters and in 49 instances this was less than 3 mm. Cankers developed at 45, or 59 per cent, of the leaf

scars where infected petioles had been attached, and at 38, or 77 per cent, of the scars where the petiole lesion had been less than 3 mm. distant from the abscission layer. One canker developed where the petiole lesion was 4 mm. distant, and one where this distance was 7 mm. A total of 48 leaf scar cankers developed, of which 45, or 94 per cent, appeared where an infected petiole had been attached. Two of the other 3 cankers developed at scars where the leaf had fallen before September 27. It has been observed that leaves with basal petiole lesions frequently fall prematurely.

It has also been noted that there is some correlation between the percentage of petiole infection in different trees and the number of cankers per twig observed the next year. For example, in one tree showing 15 per cent petiole infection in 1920, there later appeared 18 cankers per 100 twigs, and in another tree showing 24 per cent petiole infection, there later appeared 30 cankers per 100 twigs. One tree showing 15 per cent petiole infection in 1921 later developed 54 cankers per 100 twigs, another tree with 41 per cent petiole infection developed 137 cankers per 100 twigs, and a third showing 72 per cent petiole infection developed 400 cankers per 100 twigs.

These observations show that there is a close correlation between basal petiole infection and the development of leaf-scar cankers.

INTERNAL INVASION OF LEAF SCAR

While the petiole lesion may be so close to the abscission layer that it visibily extends across to the twig, usually this is not the case (Pl. 1, G, H), and the question at once arises as to the exact manner in which the fungus gains entrance to the twig.

Cultural tests in the fall of 1921 with a number of Northwestern twigs bearing leaves with petiole lesions have indicated how this invasion occurs. The clasping bases of 36 petioles bearing basal lesions were cut off 1 or 2 mm. below the margin of the lesion, sterilized a few minutes in a mercuric chlorid solution, rinsed, and planted in poured plates of potato dextrose agar. The blotch fungus developed from 19, or 53 per cent, of these leaf bases. In one successful isolation the cut was 3 mm. below the margin of the lesion. In certain cases the fungus visibly grew out from both the cut end and the callous end of the leaf base. In another test a petiole segment cut off 1 mm. below a lesion yielded the fungus while the next segment, 3 mm. below the lesion, did not yield the fungus. These tests show that the fungus may progress downward inside of the petiole tissue to a distance of 1 to 3 mm. from the visible margin of the lesion.

A number of leaf scars from which petioles with basal lesions had been removed were cut out of the twigs, sterilized a few minutes in a mercuric chlorid solution, rinsed, and planted in agar plates. Although there had been no visible indication of any infection about any of these scars, 13, or 12.5 per cent, of the 104 thus tested yielded the blotch fungus in culture. In these cases the lower margin of the petiole lesion was within at least 2 mm. of the abscission layer. These tests, made the last of September, show that in a certain percentage of cases the mycelium from a basal petiole lesion grows downward inside of the petiole tissue, penetrates the abscission layer, and invades the leaf-scar tissue of the twig before the leaf falls.

TWIG INVASION FROM BUD SCALES

There is a possibility that some of the twig infection may proceed from bud scales. It has been previously mentioned that out of the 129 cankers noted on Oldenburg twigs of the current season in October, 1921, 21, or 16 per cent, were immediately below terminal buds, and had apparently resulted from bud scale infection and subsequent mycelial invasion of the twig (Pl. 1, A). No tests to prove this theory have yet been made, but the shape and location of such lesions indicate that they may originate in the manner suggested.

LIMB INVASION FROM SPURS

On the Oldenburg variety, encircling blotch cankers often occur on large limbs at nodes where spurs have developed from dormant buds (Pl. 3, A). Whether such a canker is the result of leaf-scar invasion at the time a leaf was borne at that point or the result of a much more recent invasion from a younger canker on the spur, a suggestion made by Mr. R. A. Simpson, is not easily determined. Because of their position in the lower interior part of the tree, these spurs are highly subject to petiole infection and subsequent canker formation. However, cankers are very common at nodes where no spur has developed and must have developed from petiole infection or direct spore infection when that portion of the limb was bearing leaves. It seems likely that both types of limb infection occur and that, if no spur has developed, the canker is very nearly as old as the limb at that point, whereas, if a spur is present, the age of the canker is uncertain.

LONGEVITY OF FUNGUS IN CANKERS

Cultural tests have proved that the blotch fungus may remain alive many years in the advancing margin of the canker where it may continue to produce pycnidia and spores. The diseased tissue is very rapidly invaded by other fungi, but portions of the extreme edge of the purple advancing margin cut out with a scalpel, sterilized a few minutes in a mercuric chlorid solution, rinsed, and planted in agar plates yielded cultures of the blotch fungus, as shown in Table I.

TABLE I.—*Longevity of blotch fungus in cankers*

Variety.	Age of wood.	Number of cankers tested.	Number yielding blotch fungus.
	Years.		
Oldenburg	3	8	5
Do	4	4	4
Do	5	6	2
Transparent	6	3	2
Oldenburg	7	4	4
Do	8	2	2
Do	14	2	2

Possibly the cankers on the limb 14 years old may have resulted from infected spurs. In the other cases, however, it seems certain that the

age of the canker was about the same as that of the wood. Spore-bearing pycnidia were found in the margins of the cankers on the limbs 7 and 8 years old, and it is apparent that the fungus may remain active in the cankers many years. Anderson (*3, p. 389*) has recently reported similar findings in Illinois.

SPRAY CONTROL OF LEAF AND TWIG INFECTION

By means of spray tests conducted primarily for a study of control of fruit infection and carried out in cooperation with Laurenz Greene and C. E. Baker, of the Purdue University Agricultural Experiment Station, and with certain orchardists, the effect of the blotch sprays upon petiole and twig infection has been ascertained. The control of leaf infection was determined by counts made in the fall at the time of harvesting the apples, but the effect upon canker formation could not be determined until a year later. A more complete account of these spray tests has been published in a station bulletin (7).

Considerable interest has been aroused among growers in the possibility of controlling blotch by means of dormant sprays of concentrated strength such as lime sulphur, 1 to 3. Cultural tests made in 1919 showed that the spores already present in the pycnidia were killed by a dormant spray of lime sulphur, 1 to 3. Guba (*9*) in Illinois likewise found that the spores in the pycnidia were killed by lime sulphur, 1 to 3, 1 to 5, 1 to 6, and 1 to 8, as well as by copper sulphate, 1 to 6, but observed that the cankers continued to enlarge and produce spores. Extensive field tests in Indiana have shown that dormant sprays have no effect whatever on subsequent leaf infection and canker formation.

The failure of dormant sprays to prevent the development of cankers from infection already present in the leaf scars is shown by the data in Table II, obtained on Northwestern trees at Knightstown. The sprays were applied in the spring of 1921 and the canker counts were made on the 1920 wood.

None of the dormant applications prevented the development of cankers on the sprayed twigs. That dormant sprays have no influence on subsequent leaf and twig infection is shown in the data included in Tables III and V.

The effect of blotch sprays applied two, four, and six weeks after petal-fall in 1920 upon leaf and twig infection in Northwestern trees is shown in Table III. The data on twig infection were obtained the following year. All except the first two trees received a dormant spray of lime sulphur, 1 to 8.

TABLE II.—*Effect of dormant sprays on canker development*

Tree.	Dormant spray.	Twig infection.		
		Number examined.	Percentage infected.	Cankers per 100 twigs.
2R5	Lime sulphur, 1 to 8	103	14.6	18.4
3R2	Lime sulphur, 1 to 3	112	61.6	137.0
2R2	Lime sulphur, 1 to 3	102	62.8	115.0
2R6	Copper sulphate, 1 to 200	142	21.9	30.3

TABLE III.—*Spray control of leaf and twig infection, Knightstown, 1920*

Tree.	Sprays applied 2, 4, and 6 weeks after petal-fall.	Petiole infection.		Twig infection, 1920 wood.		
		Number examined.	Percentage infected.	Number examined.	Percentage infected.	Cankers per 100 twigs.
2R2	None (L. S. 1 to 3, dor.) [a]	757	36	102	63	115
3R2	None (L. S. 1 to 3, dor.)	697	39	112	62	137
2R5	None	1,101	15	103	15	18.4
2R6	None	1,063	24	142	22	30.3
3R3	Bordeaux 4-6-50	954	0.6	114	1.8	1.8
2R4	do	1,057	0.1	120	0.8	0.8
6R4	do	1,049	0.4	110	0	0
2R7	do	1,360	0.2	100	0	0
8R7	do	1,124	0.2	117	0	0
3R8	Lime sulphur 1 to 40	1,037	0	129	0.8	1
9R8	do	1,220	0	110	0.9	1

[a] Lime sulphur, 1 to 3, dormant.

The results in Table III show that the blotch sprays applied 2, 4, and 6 weeks after petal-fall gave a very perfect control of petiole and twig infection, and that twig infection is strictly correlated with petiole infection. The importance of the latter consequently becomes apparent. The control of fruit infection, to be presented in another publication, was in all cases comparable to the control of petiole infection. Lime sulphur appeared to be as effective as Bordeaux, but the following year it proved less so. The dormant spray of lime sulphur 1 to 3 exerted no control whatever.

The effectiveness of the blotch sprays against twig infection is further attested by the fact that cankers were very abundant on the 1919 wood of the same trees in which practically none appeared on wood formed in 1920, the year the blotch sprays were first applied.

At Mooresville, in 1921, information was obtained relative to the ineffectiveness of dusts in controlling petiole infection. These results are presented in Table IV. These results indicate that dusting failed to prevent leaf infection.

TABLE IV.—*Dusts versus spray, Mooresville, 1921*

Applications 2, 4, and 6 weeks after petal-fall.	Petiole infection.	
	Number examined.	Percentage infected.
Sulphur dust	283	35
Bordeaux dust	184	50
Bordeaux spray	2,067	4
None	611	94

The effectiveness of Bordeaux (3-5-50) blotch sprays against petiole infection was noted by C. L. Burkholder on Ben Davis at Solon in southern Indiana. Out of 893 leaves from unsprayed trees, 88 per cent showed petiole infection, and none of the 2,182 leaves from sprayed trees showed any infection.

The trees at Knightstown recorded in Table III received the same sprays in 1921 and the results are shown in Table V. It should be noted that the twig infection was, of course, determined the following year.

TABLE V.—*Spray control of leaf and twig infection, Knightstown, 1921*

Tree.	Sprays applied 2, 4, and 6 weeks after petal-fall.	Petiole infection.		Twig infection, 1921 wood.		
		Number examined.	Percentage infected.	Number examined.	Percentage infected.	Cankers per 100 twigs.
2R2	None (L. S. 1 to 3 dor.)	771	72. 4	101	93	400
3R2	do	1, 154	68. 8	101	82	546
2R5	None	1, 353	15. 1	102	30	54
2R6	None ($CuSO_4$, 1 to 200, dor.).	1, 210	41. 3	100	38	137
3R3	Bordeaux, 4-6-50	1, 340	0. 07	100	0	0
2R4	do	1, 349	0	100	0	0
6R4	do	1, 234	0. 08	100	0	0
2R7	do	1, 283	0. 8	100	0	0
8R7	do	1, 426	0. 14	100	0	0
3R8	Lime sulphur 1 to 40	1, 599	10. 9	106	2. 8	4. 7
9R8	do	1, 097	5. 3	100	5	5

The results shown in Table V indicate that the Bordeaux blotch sprays controlled petiole and twig infection almost perfectly in 1921, while lime sulphur proved distinctly less effective. The dormant sprays did not influence the disease in the least.

The control trees were infected much more seriously than in 1920, but when compared with each other, show the same relative degree of infection as in 1920, a condition to which reference will be made later.

In 1921 lime sulphur 1 to 40 and two strengths of Bordeaux were tested on Oldenburg at Mitchell, with results as presented in Table VI. All except the control trees received a lime sulphur spray at petal-fall.

The results in Table VI show that the weaker Bordeaux as well as the standard strength effectively prevented petiole infection, while the lime sulphur was distinctly unreliable.

In 1922 the two weeks spray was applied three days late at Knightstown and the control of petiole infection was rather poor, as shown in Table VII.

TABLE VI.—*Spray control of petiole infection, Oldenburg variety, 1921*

2, 4, and 6 weeks' spray.	Petiole infection.	
	Number examined.	Percentage infected.
None	1, 206	93. 0
Bordeaux 4-6-50	1, 120	3. 3
Bordeaux 2-4-50	1, 245	1. 7
Lime sulphur 1°	1, 196	26. 0

TABLE VII.—*Spray control of petiole infection, Northwestern variety, 1922*

Tree.	2, 4, and 6 weeks' spray.	Petiole infection.	
		Number examined.	Percentage infected.
4R1	None	1,082	80
5R1	do	1,017	88
3R2	Bordeaux 4–6–50	1,230	31
3R3	do	1,179	12
2R5	Bordeaux 2–4–50	1,141	37
2R7	do	1,120	9
2R6	Lime sulphur 1°	995	46
3R8	do	1,042	45

Despite the poor control with Bordeaux, its superiority over lime sulphur is apparent, and the equal effectiveness of the weaker strength is again manifest. Results obtained on Oldenburg fruit in southern Indiana proved that blotch infection started prior to two weeks after petal-fall in 1922 and proved the necessity of an earlier blotch spray in seasons such as 1922, when the blossoming period is prolonged and petal-fall occurs late.

The results presented above show that the Bordeaux sprays which are effective against fruit infection likewise prevent petiole infection, and as a consequence prevent twig infection and canker formation. In this capacity, lime sulphur is not as effective as Bordeaux, but a weaker Bordeaux seems as satisfactory as the standard strength. Under certain conditions an additional spray earlier than two weeks after petal-fall is necessary.

Scott and Rorer (*15*), Roberts (*12*), and others have noted that the sprays which prevent fruit infection also prevent canker formation to a certain degree. These results show how this is brought about and emphasize the necessity of spraying every year, regardless of crop, as Anderson has urged (*2, p. 26; 3, p. 385*), in order to prevent petiole infection and consequent canker formation. Owing to the longevity of the fungus in the cankers, it is probable that it will be necessary to spray continuously for at least seven or eight years to eradicate the fungus from the tree, and one year omitted will result in a crop of new cankers that will necessitate starting the whole campaign over.

DISTRIBUTION OF CANKERS IN OLD ORCHARDS

While a large number of varieties are susceptible to fruit infection, fewer are subject to abundant canker formation and the latter varieties are especially important as harborers and carriers of the disease. Northwestern and Oldenburg are conspicuous examples of this class in Indiana, and also in Illinois according to Anderson (*1*). Among the very susceptible varieties, Benoni, Akin, Missouri, Mann, and Willow are more subject to canker formation than Ben Davis and Stark. Rome and Transparent seem to be more subject to canker formation than to fruit infection. Cankers have been noted sparingly on Grimes, Wealthy, Stayman, Champion, Gideon, Rambo, and Salome, but have not been noted on York, Jonathan, Arkansas, and Winesap, on which varieties occasional fruit infection occurs.

It has been observed among trees of the same variety in the same orchard that blotch is more severe in some than in others and that this relative difference between individual trees remains more or less constant year after year. This phenomenon is illustrated by the data of 1920 and 1921 on four unsprayed Northwestern trees, presented in Table VIII.

TABLE VIII.—*Individuality of trees in degree of infection*

Tree.	Percentage of petiole infection.		Cankers per 100 twigs.	
	1920	1921	1920 wood.	1921 wood.
2R2	36	72.4	115	400
3R2	39	68.8	137	546
2R5	15	15.1	18	54
2R6	24	41.3	30	137

The disease was worse on all trees in 1921 than in 1920, but was consistently worse in the first two trees than in the others and was least severe in the third tree. The correlation is most striking in the canker infection of the two years. In 1920 the relative prevalence of cankers in the four trees may be expressed as 100–119–16–26 and in 1921 as 100–136–13–34. There is a marked individuality of trees with regard to the severity of blotch.

In individual trees it has been noted that cankers are much more prevalent in the lower than in the upper part of the tree, a condition which would naturally result from drip infection in a water-disseminated fungus of this type. In very badly diseased Northwestern trees, only a very few cankers could be found in the topmost branches. The highly susceptible watersprouts and suckers, because of their position in the tree, are subject to abundant infection and should of course be pruned out every year, as Scott and Rorer (*15, p. 21*) and others have recommended. It is impossible to prune out the cankers on bearing wood because of their prevalence in old trees.

CANKERS IN YOUNG ORCHARDS

Because of the large and increasing acreages of young commercial orchards in Indiana, new aspects of blotch control have developed. Blotch cankers have been found very commonly present in young trees of such varieties as Oldenburg, Transparent, and Benoni. In a block of trees at Vincennes, set out in 1918, 98 out of 156 Oldenburg trees, or 63 per cent, and 53 out of 61 Transparent trees, or 88 per cent, bore blotch cankers. The infected trees were scattered here and there and almost all infection was in the form of old cankers on the trunks or large branches. The blotch sprays had been applied during the preceding two years, so practically no young cankers had been formed. There was no evidence of tree-to-tree spread of the disease and all indications were that the original nursery stock had been infected.

In a block of interplanted 2-year-old Ben Davis and Oldenburg trees, 19 out of 36 Oldenburg trees showed blotch cankers while none were noted on the Ben Davis trees. In the same plantation, 3-year-old Oldenburg trees from another source showed very little blotch. In a

block of 53 Rome trees near Paoli, 27 contained blotch cankers. In all these cases the evidence pointed toward introduction of blotch with the nursery stock.

The cankers in young trees were always in the lower parts of the tree. In trees planted in 1917 none were over 6 feet from the ground, but in trees planted in 1915 some cankers occurred considerably higher.

CANKERS ON NURSERY STOCK

M'Cormack (*11, p. 135*) in 1910 reported blotch on nursery stock in Indiana, and Melhus, as reported by Curtiss and Brown (*4, p. 30*), in Iowa has recently found first-year apple grafts seriously injured by blotch and has also found seedlings infected. The occurrence of 159 blotch cankers on the first-year scion wood of 10 trees received from an Oklahoma nursery has been mentioned. Old ankers were also present on the stock below the graft (Pl. 2, C). Blotch cankers have been found on 1-year-old Benoni trees in a nursery row and also on the trees from which the Benoni buds were obtained. Cankers have been found in great abundance on apple seedlings imported from Kansas for budding purposes. After these were budded, cankers occurred both above and below the inserted buds (Pl. 2, A, B) and even on the underground root-bearing portions of the stem.

Nurserymen should guard against blotch by rejecting infected seedlings, and by taking bud-sticks only from sprayed or blotch-free trees, because of the danger of the presence of invisible leaf-scar infection about the buds. The nursery row affords ideal conditions for the spread of a water-disseminated fungus such as this, and nurserymen and seedling growers should apply the blotch sprays annually.

Undoubtedly blotch is carried far and wide and introduced into young orchards with the nursery stock and, as Anderson (*1; 3, p. 389*) has advised, orchardists should reject shipments containing infected trees.

CANKER ERADICATION IN YOUNG ORCHARDS

The facts presented relative to the presence of blotch on nursery stock and on scattered trees in young orchards, as well as the greater incidence of cankers in the lower parts of the trees and the constant differences in severity of the disease in individual trees, assist one in interpreting the progress of the disease in an orchard. Starting with the trees which were originally infected in the nursery, it would seem that the disease slowly progresses upward by the splashing of spores from the older cankers to leaves higher up until by the time the tree comes into bearing, there are cankers high enough to provide a "cone" of drip and splash infection inclusive of most of the tree. Even though considerable tree-to-tree spread of infection occurs in old orchards, this appears to be uncommon while the trees are young and widely separated, and, to a certain extent, each tree continues to present a more or less independent pathological picture in which the severity of the disease depends upon the early presence of cankers and their subsequent rate of multiplication. Furthermore there would seem to be little, if any, dissemination from one orchard to another.

On the basis of this theory, the elimination of the disease from a young orchard should greatly reduce, if not entirely obviate, the future blotch menace in that orchard. Such a result may be accomplished, it is believed, by two measures, the annual application of the blotch sprays

at least for a few years to prevent the formation of new cankers, as Anderson (*2, p. 26; 3, p. 385*) has recommended, and the eradication of the old cankers by pruning and excision. The effectiveness of the sprays in canker prevention has been shown, and the recommendation of Scott and Rorer (*15*) and others that cankered twigs be pruned out is particularly applicable in the case of young trees.

The feasibility of canker eradication in young trees has been tested in cooperation with Mr. R. A. Simpson at Vincennes, Ind., and it has proved to be a comparatively simple process. Many cankers were removed by pruning out spurs and smaller limbs, and the cankers on the trunks and larger limbs were cut out with a sharp knife. The fungus does not penetrate much more than halfway through the bark and the diseased bark tissue can be shaved off without cutting deeply enough to injure the underlying cambium layer (Pl. 3, A, B). A number of cankers were excised from Oldenburg trees in the spring of 1921 and the wounds healed rapidly and perfectly. A few cases of marginal renewal of fungous growth showed that it is necessary to cut about 1 cm. in advance of the visible margin of the canker in order to remove all of the mycelium. This is particularly true at the sides since the canker tends rapidly to encircle the limb (Pl. 3, A). It is also essential to cut deeply enough to remove all of the discolored tissue.

In a block of Oldenburg and Transparent trees set out in 1918, the blotch cankers were removed early in April, 1922. Cankers were found in 58 of the 156 Oldenburg trees and from these about 59 cankered limbs or spurs were pruned out and 134 cankers were excised. Cankers were found in 48 of the 61 Transparent trees, mostly on the trunks, and from these trees three cankered limbs were pruned and 145 cankers shaved off. Since this plot had received the blotch sprays the previous year, very few new cankers appeared.

Reinspections of this plot early in November and in April, 1923, showed that 40 per cent of the trees operated upon were free from cankers. The wounds had become mere surface scars in the bark and no injury to the trees was noticeable (Pl. 3, C). A coating of melted paraffin had been applied to some of the wounds but proved to be unnecessary. However, about 300 cankers scattered among 110 trees had been overlooked and were removed. The need of more than one reinspection and repetition of the process is apparent. Fire blight was prevalent when the blotch cankers were cut out, but no cases of infection of the cuts were found.

In a smaller block of 34 Oldenburg trees planted in 1920, 19 bore cankers and from these trees 61 cankers were removed on April 26. On November 9, 50 per cent of the trees operated upon were free of infection, but four cases of marginal renewal of fungous growth and 44 cankers were found. Most of the latter were on 1921 wood, the result of a failure to apply the blotch sprays in 1921, and were not visible in April when the first inspection was made. This emphasizes further the necessity of a reinspection to detect cankers overlooked or invisible at the time of the first operation.

In an orchard near Paoli, Ind., 27 out of a group of 55 young Rome trees contained cankers and from these trees 23 cankered limbs were pruned and 75 cankers were shaved off. In a block of 24 Transparent trees about 10 years old, 20 bore cankers and from these trees 92 cankered limbs were pruned and 116 cankers excised. By fall these excision wounds were mere scars in the bark.

In April, 1922, canker eradication was carried out on an extensive scale by Mr. Simpson in an orchard containing about a thousand Oldenburg trees planted in 1917 and sprayed for blotch in 1920, 1921, and 1922. In November 214 of these trees were examined (Pl. 3, C) and it was found that more than half of the 152 trees operated upon were free from infection. Cankers had been overlooked in 78, or about one-third, of the trees and a very few cases of marginal renewal of fungous growth were found. However, the great majority of the cankers had been removed and one or two repetitions of the process should practically eliminate the disease since the sprays have prevented the formation of new cankers.

Other owners of young orchards in the Vincennes region have cut the cankers from their trees and the method bids fair to be widely adopted. A Kansas orchardist, William Freienmuth (5), recently reported success in cutting out blotch cankers in a 20-year-old orchard of Ben Davis and Missouri and strongly advocates canker eradication. He began his work in the fall of 1919 and employed somewhat the same methods advised above except that the wounds were treated with a Bordeaux wash.

It seems advisable to cut out cankers early in the spring because the absence of leaves facilitates detection of the cankers and because the wounds heal most rapidly at that time. No disinfectant or wound dressing has been necessary on young trees. This operation is harmless and inexpensive and removes a dangerous source of blotch infection in young orchards.

SUMMARY

(1) Blotch cankers on bearing wood are usually located at leaf scars. On suckers, watersprouts, nursery stock, and seedlings, cankers occur at and also between the leaf scars. Cankers also occur at the bases of terminal bud scales and at the bases of spurs.

(2) Cankers at the leaf scars as a rule become visible during April and May of the second season in central Indiana.

(3) Basal petiole lesions are extremely prevalent in badly diseased trees.

(4) Cases have been noted in which the basal petiole lesions visibly extended across the abscission layer to the twig tissue.

(5) Cankers appear at leaf scars to which leaves with basal petiole lesions were attached, particularly where the edge of the petiole lesion was not more than 2 or 3 mm. from the abscission layer.

(6) Cultural tests have shown that the fungus from a basal petiole lesion grows down inside the petiole to a distance of 2 or 3 mm. and even farther and in many cases crosses the abscission layer into the twig tissue before the leaf falls.

(7) Cankers may originate from terminal bud-scale infection, and cankers on large limbs about the base of a spur may have developed from a canker on that spur.

(8) In general, the age of the canker is only slightly less than that of the wood in which it is located. The blotch fungus has been found alive in the margins of cankers on wood up to 7 and 8 years old and in one case on wood 14 years old.

(9) The blotch sprays which prevent fruit infection also prevent petiole infection and as a consequence canker formation. The prevalence of cankers is proportional to the amount of previous petiole infection.

(10) Dormant sprays of concentrated lime sulphur (1 to 3) did not influence canker development, although spores already present in the pycnidia may have been killed. Subsequent petiole infection was not checked.

(11) Sulphur and Bordeaux dusts were unreliable in the prevention of petiole infection and canker formation.

(12) Bordeaux 4–6–50, and 2–4–50 as well, applied 2, 4, and 6 weeks after petal-fall prevented petiole infection and subsequent canker formation. Under certain conditions an earlier spray application is necessary. Lime sulphur 1° or 1 to 40 was not as reliable as Bordeaux.

(13) In old orchards certain varieties are more severely cankered than others and are harborers of the disease. Cankers are most abundant in the lower parts of the tree. Individual trees show more or less constant differences year after year, in the degree of infection.

(14) Cankers have been found on a considerable percentage of the trees in young orchards, especially Oldenburg, on the trunks and older limbs. The infected trees were scattered and there was evidence of disease introduction with the nursery stock and very little evidence of tree-to-tree spread in young orchards.

(15) Cankers have been found in great abundance on nursery stock on both stock and scion and on seedlings used for budding purposes. The evidence indicated that the nursery row afforded ideal conditions for the spread of blotch.

(16) It has proved feasible to eradicate the blotch cankers from young orchards by pruning cankered spurs and small branches and by excising the cankers on the larger limbs. The cankers are shallow and can be shaved off with a sharp knife in the early spring without injury to the underlying cambium. To remove all the mycelium the cuts must be deep enough to remove all the discolored tissue and must extend about 1 cm. beyond the visible margin of the canker. Healing occurs rapidly and no disinfectant or wound dressing has been necessary.

(17) By canker eradication and annual application of the blotch sprays to prevent the formation of new cankers, it seems entirely possible that the future blotch menace in young orchards may be avoided.

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PLATE 1

A.—Apple-blotch canker at base of terminal bud scale.
B.—Canker between leaf scars on watersprout.
C, D, F.—Cankers at leaf scars on bearing wood.
E.—Leaf-scar canker on nursery stock.
G, H, I.—Basal petiole lesions. In I the lesion has visibly crossed to the twig.

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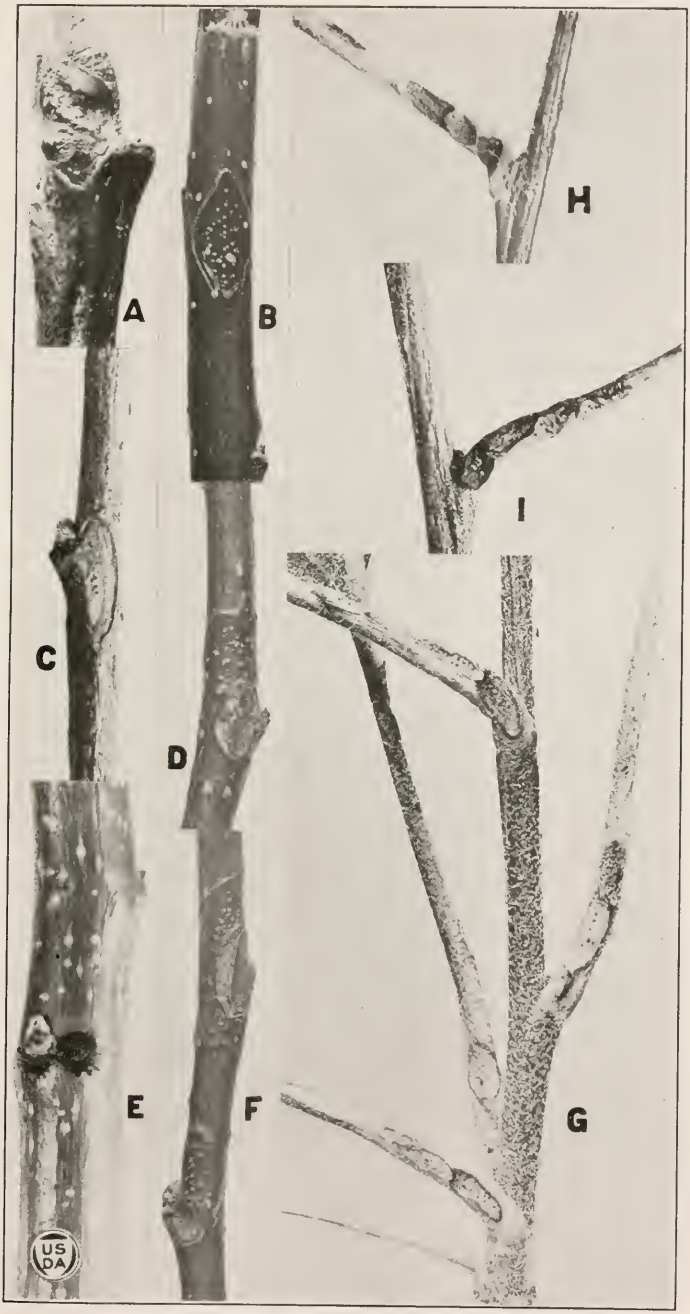


A
B
C

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PLATE 2

A.—Apple-blotch cankers on a budded seedling below the inserted bud.
B.—Cankers on a budded seedling, above and below the inserted bud.
C.—Cankers on a nursery tree above and below juncture of stock and scion.

PLATE 3

A.—Encircling type of apple-blotch canker on Oldenburg at a node.

B.—Canker excision. Canker shown in A, cut out with a sharp knife. The cut is not deep enough to injure the cambium.

C.—Results of canker excision in young Oldenburg tree. Cankers cut out in April, photographed in November. Wounds healed perfectly. Paraffin coating was unnecessary. One canker was overlooked, as indicated by the fresh cut.

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DETERMINATION OF THE SURFACE AREA OF CATTLE AND SWINE [1]

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HISTORICAL

The modern conception of nutrition may very properly be ascribed to the classic investigations of Lavoisier (*6*).[3] He recognized that the production of animal heat is an oxidation process, and conducted experiments to demonstrate his views.[4] It was many years before it became established that the oxidative processes alone could entirely account for all the heat produced by the animal body. In the meantime, however, rough comparisons were made of the heat produced by different individuals, and it soon became apparent that the amount of heat produced under comparable conditions was not proportional to the weights of the subjects investigated.

It is sufficient for the present to say that as yet no entirely satisfactory method has been devised for the comparison of heat production of animals of different sizes, but in 1848 Bergmann (*3*)[5] made a suggestion that has found wide application. He expressed the belief that the relatively high heat production of small animals per unit weight is due to their relatively greater surface area. This view was given strong support by the classic researches of Regnault and Reiset (*10*). Their studies were concerned largely with the respiratory exchange, as manifested by various species under diverse conditions. They believed that the chemical changes within the body are so complicated that it would be impossible to calculate the resulting heat production (*10, p. 513*). As a result of the observations of these collabora however, it was established that the oxygen consumption of animals is not proportional to their weights, and that the smaller the animal the higher the oxygen consumption per unit weight (*10, p. 473*). The sparrow, for example, in unit time consumes nearly 10 times as much oxygen per kilogram live weight as a fowl. Obviously, the heat production per kilogram would have a similar ratio. Their explanation was that the smaller animals have a relatively greater surface area. While their reasoning on that point is faulty, at least in part, their statement of the facts is substantially correct.

Apparently, no actual measurements of the surface area of living beings were made until 1879, when Meeh (*8*) published such measurements on man and suggested that the surface area of any individual could be calculated by the use of a formula. His formula is based on the mathematical relation that exists between the surfaces and volumes of similar

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[2] The data for this paper were taken from the thesis of Charles I. Skouby, presented at the University of Missouri in partial fulfilment of the requirements for the degree of master of arts.

[3] Reference is made by number (italic) to "Literature cited," pp. 429-430.

[4] Cited by Lusk (*7, p. 33*).

[5] Cited by Benedict (*2*).

solids—that is, the surfaces are proportional to the two-thirds powers of their volumes. If the specific gravity were the same in each case, then the surface areas would also be proportional to the two-thirds powers of the weights. The formula of Meeh may be expressed as follows: $S = KW^{2/3}$. S is the surface area in square centimeters, W is the weight in grams, and K is the constant 12.312 for adults and 11.9 for children. According to Meeh's calculation the extreme variations were ± 7 per cent.

A few years later Rubner (*11*) measured the surface area of a number of dogs and determined their heat production under comparable conditions. All the animals were mature, in the condition of inanition, and the surrounding temperature was approximately the same. The heat produced is given in 24-hour periods, measured at a temperature of 15° C.

TABLE I.—*Heat production in relation to body weight and surface area*

No. of dog.	Average body weight.	Calories liberated—	
		Per kilo body weight.	Per square meter surface area.
	Kgm.		
I	31.20	35.68	1,036
II	24.00	40.91	1,112
III	19.80	45.87	1,207
IV	18.20	46.20	1,097
V	9.61	65.16	1,183
VI	6.50	66.07	1,153
VII	3.19	88.07	1,212

The heat production per kilo varied widely, but was quite constant per square meter of body surface.

Some years later Rubner (*12*) conclusively demonstrated that the oxidation processes within the body are alone sufficient to account for the entire heat production. He constructed a respiration calorimeter and made seven experiments on dogs. These covered in all a period of 45 days, and the average heat production determined directly from the calorimeter differed from that computed from the respiration apparatus by less than one-third of 1 per cent.

Thus, after the lapse of more than one hundred years following the original pronouncement of Lavoisier (*6*), it was conclusively demonstrated that the oxidation processes alone could account for all the animal heat produced. It was shown clearly that the quantity of heat produced was not proportional to the weights of the animals, but it became reasonably certain that the quantity of heat does bear some direct relation to the surface area of the subjects investigated. Accordingly, it has become the practice of many investigators when comparing the energy transformations of animals of different weights, to compare the heat production per unit of surface area.

Before dismissing the historical phase of the subject, mention should be made of the fact that Benedict (*2*) has sharply challenged the practice of calculating heat production per unit of surface area, and concludes "that the metabolism or heat output of the human body, even at rest, does not depend on Newton's law of cooling and is therefore not proportional to the body surface." Benedict's statement applies of course

to the animal body as forcefully as to the human body. The heat output does not depend on Newton's law of cooling and it is not proportional to the body surface under all circumstances. In the opinion of the writers, however, this method of computing energy transformations has great advantages over any hitherto proposed.

Whether Benedict's objection is valid or not, in practice the animal husbandryman has some difficulty in comparing the metabolism of his experimental animals per unit of surface area. The proper constants have been determined in only a few instances, so it has been impossible to calculate the surface areas of the ordinary farm animals with any degree of accuracy. That difficulty was avoided, however, by making the calculations on the basis of a uniform weight, as was the practice of Armsby (*1, p. 260*). According to the Meeh formula, this means that the surfaces of animals are proportional to the two-thirds powers of their weights. If the formula is correct, that method would be satisfactory. As a matter of fact, it soon became evident that the formula may give very erroneous results. Trowbridge, Moulton, and Haigh (*14*) published a number of measurements of the surface area of cattle, and calculated the constant for the Meeh formula, expressed as follows:

$$K = \frac{S}{W^{\frac{2}{3}}}$$

Of the cattle investigated, the variations were extreme, and the constants varied from 7.319 to 10.474. The constant was greatest for the thinner and younger animals and least for those older and fatter.

Some time later Moulton (*9*) developed two formulae, differing somewhat from that of Meeh. The formula for fat cattle is:

$$A = 0.158\ W^{\frac{5}{9}}$$

and for other animals:

$$A = 0.1186\ W^{\frac{5}{8}}$$

A is the surface area in square meters, W is the empty weight in kilograms.

The work of Trowbridge, Moulton, and Haigh (*14*) and of Moulton (*9*) makes it clear that a simple formula of the Meeh type is not applicable to cattle. At about the same time that their work was published, E. F. and D. Du Bois (*4*) and collaborators had reinvestigated the formula as applied to man, and showed that it leads to serious errors. They (*5*) finally devised their "height-weight" formula which seems to be quite exact. The formula is:

$$A = W^{0.425} \times H^{0.725} \times C$$

A is the surface area in square centimeters, H the height in centimeters, W the weight in kilograms, and C the constant 71.84. The error was estimated to be ± 5 per cent as a maximum. The error with the Meeh formula as applied to their subjects (*13*) ranged from 4.9 to 38.0 per cent.

It is evident from the work of the Du Boises on man and of Moulton on cattle that the surface area can not be accurately calculated as a power function of the weight. Accordingly, we have attempted to devise a formula that would permit a more accurate calculation of the surface area of cattle and swine. The "height-weight" formula of Du Bois was taken as a guide.

EXPERIMENTAL

We wished to know the surface area of some of our experimental steers, under observation at the time, so our first measurements of cattle were made on living animals. The first step was to prepare an inelastic mold of one-half the body, that is, of one side. This was accomplished by pasting strips of strong manila paper, gummed on one side, to the hair of the animal, and building up on this foundation until the mold was rigid enough to be handled. Before it was removed the median line was marked so that when trimmed the mold represented exactly one-half the surface area. The mold was usually made in four parts. Each leg made up a part, the shoulder, neck, and head made up another, and the body and hind quarter made up the fourth. The outside of the ear was also measured and the area multiplied by two. The steers were gentle and accustomed to being handled, so with care it was possible to obtain a very accurate mold. When dry it was cut into pieces that would lie flat. These pieces were traced on blue-print paper, and the tracings cut out and weighed. The weight of the paper per square centimeter was determined, and then it was a simple matter to calculate the surface area of the animals. Before adopting this method we assured ourselves that the paper used was sufficiently uniform for our purpose.

The method employed for measuring the surface area of the slaughtered cattle consisted in tracing the outline of the entire hide on a large sheet of paper made by pasting strips of heavy wrapping paper together. With a little care the hide could be made to lie flat and an accurate tracing obtained. The area was determined by drawing rectangles and triangles on the tracing of the hide and measuring these. This method also was used for determining the surface area of the swine. In most cases only the hide from half of these animals was measured, that from the right side of the body.

In one case we had an opportunity to compare the two methods of determining the surface area of cattle. Steer No. 528 was killed not long after his surface area had been determined by use of the mold. The hide was removed and measured, and the two methods compared.

Area determined from hide	64,344 sq. cm.
Area determined from mold	64,028 sq. cm.
Difference	316

The difference in this case is less than 0.5 per cent of either determination.

MEASUREMENTS TAKEN

A number of measurements were taken on these animals, but the only one used was the "length of body." The length of body of cattle was taken as the distance measured with a tape from the point of the withers to the end of the ischium. The length of body of swine is the distance as measured with a tape from the point of the withers to the root of the tail.

The chief difficulty encountered in using the formula lies in the uncertainty as to the exact position of the "point of withers." We attempted to use some other measurement, but failed to find one more satisfactory. It is impossible, of course, to secure any cooperation from animals in taking these measurements, and, if any portion of the neck is included, the position in which they stand affects very materially the result.

Obviously, the posture of the animal has little or no effect on the measurement we have chosen. Furthermore, we do not believe that the uncertainty as to the location of the point of withers will cause any real difficulty. The measurements from which our formula was derived were taken by several observers, and so represent the probable range of variation in the selection of those points. Our calculated results would hardly agree so closely with the measured area if these variations are of great moment. In order to indicate more clearly the location of these points they have been shown on Plate 1.

In anatomical terms the "point of the withers," as we have determined it, seems to lie directly above the juncture of the second and third thoracic vertebrae. The end of the ischium is the tuber ischii, commonly called the pin bone. The point of the withers of swine, as we have taken it, lies above the juncture of the first and second thoracic vertebrae. The root of the tail is, of course, the point where tail and body join.

WEIGHT

Besides the "length of body," also the weights of the animals were used in developing the formula. This is simply the weight of the animal taken at the time the surface area was measured. Neither food nor water was withheld from the animals before weighing.

DESCRIPTION OF ANIMALS

The cattle were in practically all cases of beef breeding variety and varied from scrubs to the purebred. They varied in age from about 6 weeks to 8 years, and in condition from very thin to very fat. The weights range from 55 to 842 kgm., and the length of body ranges from 61 to 172 cm. The data include four females, two heifers, and two dry cows.

The swine were purebreds, including Poland China, Duroc Jersey, and Yorkshire breeds. They varied in age from 3 weeks to over 3 years, and in condition from very thin to very fat. The live weights ranged from 2.5 to 178.1 kgm., and the length of body ranged from 24 to 132 cm. Both barrows and females were included. The data used in developing the formula for cattle are given in Table II and for swine in Table III.

TABLE II.—*Data used for development of formula for cattle*

No. of animal.	Live weight.	Length of body.	Surface area as measured.
	Kgm.	*Cm.*	*Sq. cm.*
1 [a]	568	156	55, 407
2 [b]	550	143	50, 583
3 [a]	486	140	47, 721
4	468	138	46, 521
5 [b]	406	132	45, 171
6	55	61	12, 466
7	131	95	23, 581
8	500	132	49, 990
528	668	172	64, 028
572	408	126	45, 176
573	318	125	41, 140
571	387	136	47, 138
574	276	125	38, 236
575	302	130	40, 236
577	511	150	55, 664
578	420	136	48, 957
579	480	150	53, 190
585	432	145	49, 046
503 [c]	271	124	36, 143
509	439	147	49, 701
197	482	152	52, 810
507	457	153	50, 175
502	506	152	51, 038
541	324	111	38, 036
527	842	162	66, 343
515	743	150	58, 846
48	809	155	62, 038
501	883	152	64, 635
592	213	129	34, 345
558	108	87	20, 189
538	181	105	29, 211
524	362	145	46, 417
500	457	153	54, 148
540	158	99	26, 068
595	265	125	36, 555
525	305	131	39, 955
523	381	136	46, 827

[a] Dry cow.
[b] Heifer.
[c] The weights and surface areas of all animals below the line were published by Trowbridge, Moulton, and Haigh (*14*).

TABLE III.—*Data used for development of formula for swine*

No. of animal.	Live weight.	Length of body.	Surface area as measured.
	Kgm.	*Cm.*	*Sq. cm.*
13B	44.9	77	10,972
60B	41.1	72	10,625
3B	75.0	87	14,759
6B	74.1	85	14,702
53B	90.0	96	16,207
12B	90.0	93	16,147
33S	101.8	100	17,384
40S	121.0	95	17,365
33B	134.0	103	19,330
10B	142.0	94	19,126
100	21.1	51	6,744
101	5.0	31	2,712
102	3.6	31	2,242
103	10.3	38	4,094
104	178.1	132	27,215
105	3.1	25	1,999
106	2.5	24	1,743
1 [a]	101.3	79	14,850
2	114.1	84	16,460
5	88.2	81	14,576
6	118.2	84	16,528
8	111.0	85	15,887
9	127.7	87	17,384
10	101.0	72	14,204

[a] We are indebted to the Department of Agricultural Chemistry for all data concerning the animals below the line.

Many of the steers described in the publication of Trowbridge, Moulton, and Haigh (*14*) were measured, and these animals, 19 in all, are included with our data. These same investigators also determined the surface area and took measurements of seven swine, and we have availed ourselves of the opportunity to use this material. Unfortunately for our purpose, the length of the body as recorded by them extended forward to the poll. In order to use their data we examined similar animals in our herd and estimated that for swine of that length the measurement of Trowbridge, Moulton, and Haigh is 15 cm. longer than ours. We have, therefore, arbitrarily deducted 15 cm. from the length of body as they measured it, in order to make their measurements comparable to ours. An element of uncertainty is thus introduced with these seven animals.

CALCULATION OF FORMULA

The method of calculation was quite simple, though the process proved to be rather laborious. The observed values of the surface area, live weight, and length of body, were put in the form of an equation.

$$S = W^x \times L^y \times K, \text{ or}$$

$$\text{Log } S = x \log W + y \log L + \log K$$

Since there are three unknowns, x, y, and K, we formed three simultaneous equations in a number of different combinations and solved for

the unknown values. We were disappointed in one respect—the values obtained for the exponents were not as uniform as we had hoped. Our final procedure, therefore, was to take the values most frequently occurring and by actual trial to determine which gave the least variable values for K. The value then assigned to K was the mean of the extremes. The equation finally chosen is:

$$S = W^{.4} \times L^{.6} \times K$$

K is 217.02 for cattle and 175 for hogs. S is the surface area in square centimeters. W is the weight in kilograms, and L is the length of the body in centimeters. This formula is, of course, identical in principle with the Du Bois height-weight formula for man.

In order to test the relative usefulness of our method, the surface areas of our animals have been calculated both by the length-weight formula and the Meeh formula. The calculations for cattle include also the formula of Moulton as developed for thin animals. His calculations were based on the empty weight, so we assumed that the formula would apply to the live weight if a suitable constant were chosen. The constant selected is 1,081. The Moulton formula for fat animals was not used because we were not certain that any of our animals were sufficiently fat to be classed in that group. It would seem that the possibility of error in judgment as to which to use is an element of weakness in the Moulton formulae, though we doubt whether it would lead to a gross error.

TABLE IV.—*Surface area as measured and as calculated for cattle*

No. of animal.	Surface as measured.	Surface as calculated.					
		$S=W^{.4}\times L^{.6}\times K_1$ [a]		$S=W^{2/3}\times K_2$ [a]		$S=W^{5/8}\times K_3$ [a]	
		Area.	Error.	Area.	Error.	Area.	Error.
	Sq. cm.	*Sq. cm.*	*Per cent.*	*Sq. cm.*	*Per cent.*	*Sq. cm.*	*Per cent.*
1	55,407	56,770	+2.5	55,692	+0.5	56,924	+2.7
2	50,583	53,193	+5.2	54,509	+7.8	55,790	+10.3
3	47,721	49,984	+4.8	50,194	+5.2	51,638	+8.2
4	46,521	48,812	+4.9	48,947	+5.2	50,434	+8.4
5	45,171	44,900	−0.6	44,522	−1.4	46,148	+2.2
6	12,466	12,702	+1.9	11,743	−5.8	13,230	+6.1
7	23,581	23,444	−0.6	20,944	−11.2	22,758	−3.5
8	49,990	48,801	−2.4	51,152	+2.3	52,563	+5.1
528	64,028	64,229	+0.3	62,050	−3.1	62,996	−1.6
572	45,176	43,750	−3.2	44,668	−1.1	46,290	+2.5
573	41,140	39,411	−4.2	37,831	−8.0	39,613	−3.7
571	47,138	44,843	−4.9	43,122	−8.5	44,786	−5.0
574	38,236	37,240	−2.6	34,422	−10.0	36,257	−5.2
575	40,236	39,525	−1.8	36,551	−9.2	38,355	−4.7
577	55,664	53,152	−4.5	51,900	−6.8	53,283	−4.3
578	48,957	46,336	−5.4	45,540	−7.0	47,136	−3.7
579	53,190	51,839	−2.5	49,780	−6.4	51,240	−3.7
585	49,046	48,698	−0.7	46,403	−5.4	47,973	−2.2
503	36,143	36,791	+1.8	34,005	−5.9	35,846	−0.8
509	49,701	49,418	−0.6	46,904	−5.7	48,458	−2.5
197	52,810	52,340	−0.9	49,919	−5.5	51,372	−2.7
507	50,175	51,438	+2.5	48,177	−4.0	49,690	−0.9
502	51,038	53,366	+4.6	51,562	+1.0	52,956	+3.8
541	38,036	36,975	−2.8	38,302	+0.7	[b] *40,079*	*+5.4*
527	66,343	67,973	+2.5	72,405	+9.2	[b] *72,803*	*+9.7*
515	58,846	61,737	+4.9	66,612	+13.2	[b] *67,327*	*+14.4*
48	62,038	65,145	+5.0	70,500	+13.6	[b] *71,006*	*+14.4*
501	64,635	66,679	+3.2	74,737	+15.6	[b] *74,998*	*+16.0*
592	34,345	34,214	−0.4	28,961	−15.7	30,836	−10.2
558	20,189	20,586	+2.0	18,415	−8.8	20,170	0.0
538	29,211	28,332	−3.0	25,982	−11.1	27,853	−4.6
524	46,417	45,373	−2.2	41,245	−11.1	42,955	−7.5
500	54,148	51,437	−5.0	48,177	−11.0	49,690	−8.2
540	26,068	25,902	−0.6	23,732	−9.0	25,585	−1.8
595	36,555	36,639	+0.2	33,502	−8.4	35,347	−3.3
525	39,955	39,864	−0.2	36,792	−7.9	38,593	−3.4
523	46,827	44,564	−4.8	42,675	−8.9	44,351	−5.3

[a] Constants: $K_1=217.02$; $K_2=812$; $K_3=1,081$.
[b] These animals were very fat when measured, so this formula is not expected to apply to them. They are included merely to complete the table.

TABLE V.—*Surface area as measured and as calculated for swine*

No. of animal.	Surface as measured.	Surface as calculated.			
		$S=W^{.4}\times L^{.6}\times K$ [a]		$S=W^{2/3}\times K$ [b]	
		Area.	Error.	Area.	Error.
	Sq. cm.	*Sq. cm.*	*Per cent.*	*Sq. cm.*	*Per cent.*
13B	10,972	10,860	−1.0	9,816	−10.5
60B	10,625	10,061	−5.3	9,254	−12.9
3B	14,759	14,344	−2.8	13,818	−6.4
6B	14,702	14,081	−4.2	13,708	−6.8
53B	16,207	16,372	+1.0	15,605	−3.7
12B	16,147	16,053	−0.6	15,605	−3.4
30S	17,384	17,625	+1.4	16,933	−2.6
33S	17,365	18,314	+5.5	19,009	+9.5
40B	19,330	20,025	+3.6	20,347	+5.3
30B	19,126	19,401	+1.4	21,148	+10.6
100	6,445	6,270	−2.6	5,933	−12.0
101	2,712	2,614	−3.6	2,272	−16.2
102	2,242	2,292	+2.2	1,825	−18.6
103	4,094	3,936	−3.9	3,678	−10.2
104	27,215	26,040	−4.3	24,596	−9.6
105	1,999	1,898	−5.0	1,652	−17.4
106	1,743	1,699	−2.5	1,431	−17.9
1	14,850	15,271	+2.8	16,885	+13.7
2	16,460	16,616	+0.9	18,278	+11.0
5	14,576	14,666	+0.6	15,396	+5.6
6	16,528	16,852	+2.0	18,714	+13.2
8	15,887	16,551	+4.2	17,946	+13.0
9	17,384	17,751	+2.1	19,704	+13.3
10	14,204	14,425	+1.6	16,851	+18.6

[a] K=175. [b] K=777.

It will be noted from Tables IV and V that when the surface areas were calculated by using the two-thirds power of the weight, the maximum error with cattle is ±15.6 per cent and with swine ±18.6 per cent. The constants chosen were 812 for cattle and 777 for swine. The "length-weight" formula gives a maximum error of less than ±5.5 per cent with either cattle or swine.

The Meeh formula gives a positive error with the fat animals and a negative error with the thin ones. The errors in calculating the surface area of cattle by the length-weight formula could not be definitely correlated in any such manner with the condition of the animal. In the case of the swine the tendency of the fat animals to give a positive error and of the thin animals to give a negative error is quite distinct. We are inclined to believe this is due to the fact that as the hogs became fatter, the thickening of the subcutaneous fat pushed the root of the tail farther back, and so made the length of body measurement too long. Whatever the cause may be, we do not consider that difficulty of any importance.

The Moulton formula, which applies only to cattle, gives better results than that of Meeh, but is less accurate than the "height-weight" formula. Five of the animals, No. 541, 527, 515, 48, and 501, were very fat when the surface areas were measured, so according to Moulton the exponent 5/9 and not 5/8 should be used in these cases. The five have been included with the others, but are disregarded when making comparisons. The maximum error obtained in the remaining cases is then ±10.2. It may be suggested that some of the animals were sufficiently fat so that the exponent 5/9 and not 5/8 should be used. This may be true, but the usefulness of the formula is diminished if it is necessary to know the correct result before deciding which exponent to use.

There may be some doubt attached to the use of the Moulton formula, since it was originally derived from the empty weight of the animals. As stated previously (see Table IV), we changed the constant and applied it to the live weight. In order to make the comparison as rigorous as possible, we have applied the formula as originally devised to the empty weight of the steers described by Trowbridge, Moulton, and Haigh (*14*). These calculations appear in Table VI.

TABLE VI.—*Calculated surface areas of steers measured by Trowbridge, Moulton, and Haigh*

No. of steer.	Group and condition.	Empty weight.	Surface as measured.	Surface as calculated.			
				$S=.1186\ W^{5/8}$.		$S=.1580$ [a] $W^{5/9}$.	
				Surface.	Error.	Surface.	Error.
		Gm.	*Sq. cm.*	*Sq. cm.*	*Per cent.*	*Sq. cm.*	*Per cent.*
547	I	171,448	27,692			27,532	−0.6
541	I	288,297	38,036			36,749	−3.4
594	I, fat	247,517	32,850			33,759	+2.8
532	I	459,025	50,419			47,584	−5.6
593	I, good	317,909	38,884			38,800	+0.2
504	I	475,854	48,225			48,546	+0.7
515	I, very fat	671,917	58,846			58,801	+0.1
121	I, fat	508,513	50,104			50,369	+0.5
527	I, very fat	786,005	66,343			64,155	−3.3
513	I, very fat	772,785	61,633			63,553	−3.1
501	I, very fat	814,914	64,635			65,454	−1.3
48	I, very fat	744,708	62,038			62,260	+0.4
554	II	78,071	17,343	18,067	+4.2		
550	II	121,112	22,144	23,773	+7.4		
538	II	158,911	29,211	28,171	−3.6		
503	II	236,429	36,143	36,112	−0.1		
597	II, maintenance	302,793	42,781	42,150	−1.5		
523	II	337,803	46,827	45,134	−3.6		
507	II	418,896	50,175	51,630	+2.9		
526	II	427,995	54,751	52,329	−4.4		
197	II, good condition	444,750	52,810	53,599	+1.5		
502	II	444,424	51,038	53,575	+5.0		
512	II	493,877	60,054	57,226	−4.7		
558	III	89,999	20,189	19,746	+2.2		
540	III	137,726	26,068	25,761	−1.2		
531	III	192,005	34,083	31,707	+7.0		
591	III, very thin	190,043	33,177	31,502	−5.1		
592	III, extremely thin	187,733	34,345	31,264	−9.0		
595	III, maintenance	230,275	36,555	35,521	−2.8		
525	III	265,587	39,955	38,834	−2.8		
524	III	322,234	46,417	43,822	−5.6		
509	III	391,461	49,701	49,490	−0.4		
500	III	407,833	54,148	50,770	−6.2		

[a] This is given as 0.134 in the original paper. The author later substituted for 0.134 the constant 0.158.

It is necessary to consult the original paper to obtain a detailed description of the steers, but the following excerpt will explain the significance of the groups. "Group I was full fed and crowded. Group II was fed for maximum growth without laying on appreciable fat. Group III was fed for retarded growth—about one-half pound gain daily when yearlings." (*14, p. 6.*)

Since the formulae were derived from the weights of these animals, the circumstances are especially favorable for obtaining good results. The agreement between the calculated and observed values of the Group I animals is very close indeed. Groups II and III diverge somewhat more. Presumably, that is due to the fact that the steers in Group I were quite uniform, while those in Groups II and III varied widely in condition. In Group I the maximum errors are +2.8 per cent and −5.6 per cent. In Groups II and III the maximum errors are +7.4 per cent and −9.0

per cent. We did not have measurements of some of these animals, so were unable to apply the length-weight formula to all of them. In 19 cases, however, we have the data to make a direct comparison between the Moulton and the length-weight formulae. Reference to Tables IV and VI shows that in these instances the latter formula gives a slightly smaller maximum error and a slightly smaller average error. The maximum deviations from the measured values are +5.4 and −4.8 per cent. From the Moulton formulae it is seen that the maximum deviations are +2.8 and −7.6 per cent. The averages of all errors are, respectively, 2.5 and 3.0 per cent. This comparison hardly does more than indicate that the length-weight formula is at least as accurate as any other hitherto proposed.

With all the 37 cattle whose surface areas have been calculated by our formula (Table IV) the maximum errors were less than ±5.5 per cent. With all the 33 steers whose surface areas were calculated by the original Moulton formulae (Table VI) the maximum errors were +7.4 and −9.0 per cent. The average errors were, respectively, 2.7 and 3.1 per cent. It will be recalled (Table IV) that we modified the Moulton formula so that it could be applied to the live weight. The maximum error was ±10.2 per cent, and the average error was 3.7 per cent.

A more refined statistical method is required to properly evaluate the two methods, but we do not consider such a procedure necessary. The formula we have proposed so far as it has been tested applies to all animals of the breed, and there is no opportunity for error in deciding which formula to use. Furthermore, it is more accurate than other formulae hitherto proposed.

SUMMARY

By using the weight and length of body, a more accurate formula has been developed for calculating the surface area of cattle and swine. The formula is:

$$S = W^{.4} \times L^{.6} \times K$$

S is the surface area in square centimeters, W is the weight in kilograms, L is the length of the body in centimeters, and K is the constant 217 for cattle and 175 for swine. The maximum error is less than ± 5.5 per cent with either cattle or swine.

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PLATE 1

Method of measuring length of body of cattle and swine. The white portion of the tape indicates length of body.

430[2]

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SOIL TEMPERATURE AS A FACTOR AFFECTING THE PATHOGENICITY OF CORTICIUM VAGUM ON THE PEA AND THE BEAN[1]

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INTRODUCTION

The present paper is the third of a series of articles[2] dealing with the effects of soil temperature on the pathogenicity of *Corticium vagum* B. and C. The two earlier publications (*13*, *14*)[3] dealt primarily with the power of this organism to produce cankers on the stems of the Irish potato. In these publications results were presented from both field and greenhouse experiments which showed a definite and vital relation to exist between the temperature of the soil and the pathogenic action of *Corticium vagum* on this particular host. Such opportunity for further study of the parasitism of this organism was offered by the wide range of hosts on which the sterile or "Rhizoctonia" stage of *Corticium vagum* becomes parasitic, that studies similar to those on the potato were extended to include a number of additional hosts. The results of such studies on the pea and the bean, together with observations on the growth reaction of the fungus to temperature, are included in the following article.

APPARATUS AND METHODS

In the various pathogenicity studies the host plants were grown in metal cans 7 inches in diameter and 12 inches deep. These were submerged in a series of water jackets known at the University of Wisconsin as the Wisconsin soil-temperature tanks. These tanks, together with the methods for temperature and soil-moisture control, were described in the earlier work on the potato (*13*). Such variations in the methods and operations as were therein employed will receive consideration in relation to the individual experiments.

Temperatures given in the various tables represent the mean temperatures at which the water was maintained in each of the different tanks. Fluctuations in these temperatures did not exceed in general more than one and one-half degrees from those given in the tables, even at the extreme high and low temperatures, and were not of more than a few hours' duration. Although the surface soil in the cans was kept approximately 2 inches below the surface of the water in the tanks, the soil

[1] Accepted for publication June 25, 1923.

[2] The series of three articles on the "Pathogenicity of *Corticium vagum*" was presented to the faculty of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The writer wishes to express his indebtedness to Prof. L. R. Jones for helpful criticism and suggestions.

[3] Reference is made by number (italics) to "Literature cited", p. 449.

to a depth of 1 inch at the higher temperatures fell about 1° below that of the surrounding water. The reverse condition was found true for the soil at the lower temperatures of 9° and 12° C. This relation is of importance, as the greater amount of damage to the stems of both the pea and the bean occurred within the first 2 inches of surface soil.

In preliminary experiments, the fungus used was found to attack vigorously the tissues of the stems of both the pea and the bean. Under severe conditions, it proved to be able to attack, and even destroy, the plumule and the cotyledons of the young seedlings, and often severely damaged the secondary roots arising from the developing hypocotyl. The lesions produced on the stems were observed to vary in depth and area from mere browning and slight destruction of the epidermis, to extensive and ugly ulcers sufficiently deep practically to sever the stem. As in the earlier work with the potato, it was found that the number of stems showing injury gave no true index to the degree of damage caused by *Corticium vagum* on these plants at the various temperatures. With a view to obtaining a more accurate expression of this relation, all the diseased plants occurring at each of the various temperatures were divided into three separate classes: Slightly injured, severely injured, and cut off.[4] The percentage of plants found in these three classes were then multiplied by the units 1, 2, and 3, respectively. Quantitative values thus determined are included in the various tables under the caption, "Intensity of injury."

TEMPERATURE STUDIES WITH THE PEA

Prior to starting the temperature studies on the pea, the author had observed a series of experiments conducted by Dr. F. R. Jones with respect to the relation of soil temperature to the nature and type of lesion caused by *Corticium vagum* on this particular host. This work [5] consisted of growing five separate crops of peas in soil inoculated with the sterile stage of *Corticium vagum* at a depth of 1 inch and at temperatures of 9.5°, 12.2°, 15°, 18°, 20.5°, 23.5°, 26°, and 28° C. The first four of these experiments are interesting primarily in the fact that the steam-sterilized soil, regardless of the manner of inoculation, gave results which were so exaggerated in severity as to render them valueless for obtaining reliable temperature data. The seedlings, Jones states, were so promptly and vigorously attacked that only at the extreme high and low temperatures did the plants succeed in getting through the soil.

In growing the fifth crop Jones used unsterilized pasture soil, proved, by preliminary tests, to be free from parasitic strains of *Corticium vagum*. This he inoculated with one-fifth its weight of the infected soil used in the previous tests. Four cans for the growth of peas were maintained at each of the eight temperatures indicated in the table. Two of the cans, at each temperature, containing inoculated soil were planted with seven peas each; a third can with inoculated soil was planted with an equal number of cotton seeds; the fourth can, containing uninoculated soil,

[4] In the first group, *slightly injured*, were placed all plants showing distinct lesions but not damaged to such an extent as to interfere with continued growth. In the second class, *severely injured*, were included such plants as showed injury severe enough to definitely injure the plant in its subsequent growth. In the group, *cut off*, were included all plants with plumules destroyed and those with stems severed by the fungus subsequent to infection.

[5] The results of these experiments were compiled by Dr. Jones in the form of a report now on file in the department of plant pathology at the University of Wisconsin and are summarized here with his approval. The additional work of the author with the pea as presented here must be considered as a continuation of that begun by Dr. Jones.

was planted with seven peas and two cotton seeds and held as a control. The results as reported are graphically shown in figure 1 and in Table I. All control plants were reported to be free from lesions.

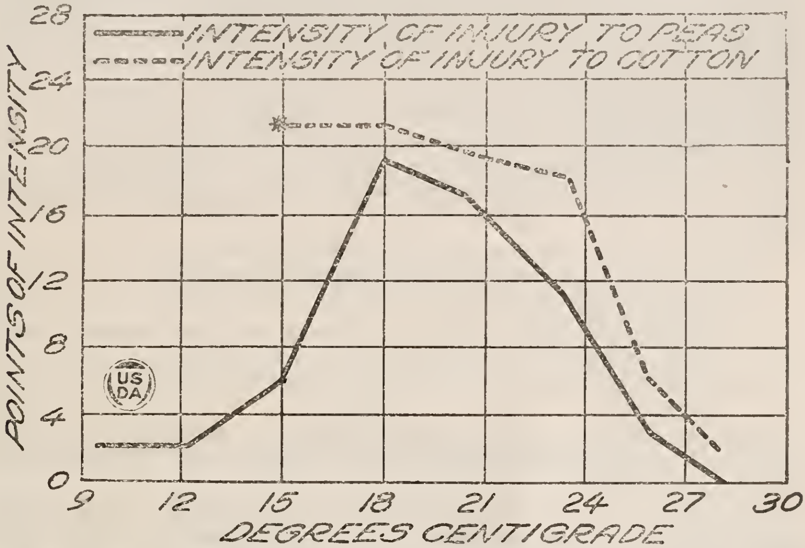

FIG. 1.—Effect of soil temperature upon the severity of injury to peas and cotton caused by *Corticium vagum.*

TABLE I.—*Effects of growing pea and cotton plants at various temperatures in soil inoculated with Corticium vagum*

PEAS

Temperature at depth of 1 inch.	Plants gro n in uninoculated soil.	Plants grown in inoculated soil.					
		Total number.	Number slightly injured.	Number severely injured.	Number cut off.	Number uninjured.	Total intensity of injury.[a]
°C.							
9.5	3	11	2	....	....	9	2
12.2	7	13	....	1	....	12	2
15.0	6	10	2	2	....	6	6
18.0	6	11	2	4	3	2	19
20.5	6	12	2	3	3	4	17
23.5	7	11	....	4	1	7	11
26.0	7	11	1	1	....	9	3
28.0	7	13	....	....	....	13	....
COTTON							
15.0	1	....	....	....	....	....	21
18.0	2	....	....	....	....	....	21
21.5	2	2	....	2	....	....	19
23.5	2	3	....	3	....	....	18
26.0	2	7	4	1	....	2	6
28.0	2	7	2	....	....	5	2

[a] In estimating the extent of injury upon the peas Jones used the following scale of values:
"Digit *1* represents a lesion on the stem which may not greatly damage the plant in its subsequent growth.
"Digit *2* represents a lesion on the stem which appears to be severe enough to greatly injure the plant in subsequent growth.
"Digit *3* represents destruction of the shoot, the root alone having made growth.
"The sum of the digits representing all the injuries found at any temperature is taken to represent the total intensity of injury at that temperature.
"In estimating the intensity of injury to cotton, the same scale was used, except that the digit *3* represents the destruction of the seed before germination. Thus the total number of seeds planted multiplied by *3* represents the total destruction of plants."

From the series of experiments Jones drew the following "tentative" conclusions:

1. The use of steam-sterilized soil inoculated with cultures of Rhizoctonia was found to exaggerate very greatly the amount of injury which the fungus causes to appear.
2. Rhizoctonia appears to do the greatest amount of injury to peas when the soil temperatures lie between 12° and 27° C. with a maximum amount of injury near 18° C.
3. Within the limits tried neither high nor low temperatures completely inhibited injury from Rhizoctonia.
4. Lesions produced by Rhizoctonia upon peas appear to be characteristic, resembling those upon potatoes.
5. It is frequently very difficult to reisolate Rhizoctonia from peas because of the presence of rapidly growing secondary invaders.
6. In a single comparison of the pathogenicity of a strain of Rhizoctonia from peas and one from potatoes no difference was observed in the nature or the intensity of the injury done.
7. Injury by Rhizoctonia to peas may be classified as follows:
   A. Destruction of the entire embryo of the germinating seed.
   B. Destruction of the primary shoot which may later be replaced by one or more secondary shoots.
   C. Production of lesions of greater or less intensity upon the stem below the surface of the ground.
   D. The early destruction of the cotyledons, thus depriving the young plant of its stored food.

In the experiments on the pea conducted by the author three successive crops were grown in the "tanks" at soil temperatures varying from 9° to 29° C.

EXPERIMENT 1.—In this experiment peas were grown at the various temperatures in unsterilized pasture soil inoculated with *Corticium vagum*[6] five days previous to planting. One can in each temperature tank was used for growing the crop in the inoculated soil. A similar series with uninoculated soil was arranged as a control. Ten seeds were planted in each can of the two series, and 2 days later the tanks were adjusted to their various temperatures. The results obtained from the inoculated soil 19 days after planting are recorded in Table II. All control plants were found to be free from lesions.

TABLE II.—*Effect of growing peas at various temperatures in soil inoculated with Corticium vagum (Experiment 1)*

Temperature at depth of 1 inch.	Number of seeds planted.	Number of plants grown in uninoculated soil.	Plants grown in inoculated soil.						
			Total number.	Number slightly injured.	Number severely injured.	Number cut off.	Number uninjured.	Percentage injured.	Intensity of injury (points).
°C.									
9.3	10	9	7	1			6	14.2	14.2
11.7	10	10	8	4	1		3	62.5	75.0
15.0	10	10	9	4	1	1	3	66.2	100.0
18.0	10	9	8	3	3	1	1	87.5	150.0
20.8	10	10	10	1	2	1	6	40.0	80.0
23.5	10	10	10	1	2		7	30.0	50.0
26.6	10	10	10				10		
29.0	10	10	10				10		

The results are especially interesting in showing the inhibiting effect of the high and low temperatures on the pathogenic action of the fungus. No damage occurred to the plants at 26° and 29° C., and plants at 9°

[6] This particular strain of *Corticium vagum* was provided by Dr. F. R. Jones, who had previously isolated it from diseased peas obtained in Wisconsin. The soil in the cans in Experiments 1 and 2 was inoculated with small quantities of soil previously inoculated for this purpose with this specific organism.

were but slightly injured. However, severe injury occurred between the temperatures of 11° and 23°. Growing-point destruction resulted only at 15°, 18°, and 21°.

EXPERIMENT 2.—In Experiment 2 plants were grown at the various temperatures in unsterilized soil inoculated with the same strain of *Corticium vagum* as that used in Experiment 1. These were checked by a similar number of plants grown in uninoculated soil. Except for the increased number of seeds planted in each pot, Experiment 2 was practically a duplicate of Experiment 1. Twelve seeds were planted in each pot in both the inoculated and uninoculated soil. The results were as shown in Table III.

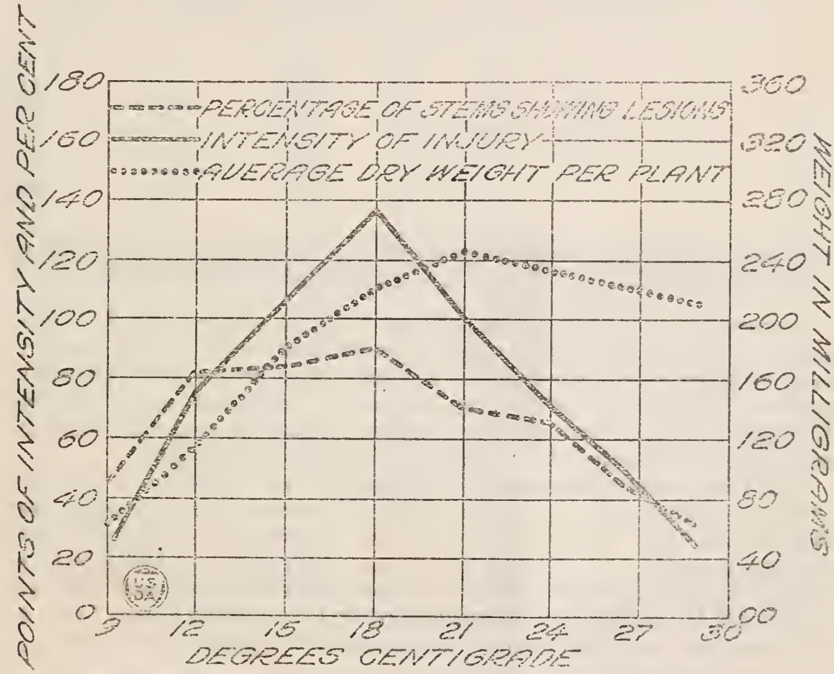

FIG. 2.—Effect of soil temperature upon the severity of stem injury caused by *Corticium vagum* and upon the growth of the pea plant as determined by the dry weight. The curve showing dry weight represents the average of the results from Experiments 1 and 2.

TABLE III.—*Effect of growing peas at various temperatures in unsterilized soil inoculated with Corticium vagum (Experiment 2)*

Temperature at depth of 1 inch.	Number of seeds planted.	Number of plants grown in uninoculated soil.	Plants grown in inoculated soil.							
			Total number.	Number very slightly injured.	Number slightly injured.	Number severely injured	Number cut off.	Number uninjured.	Percentage injured.	Intensity of injury (points).
°C										
9.2	12	10	12	9	......	......	.....	3	75	37.5
12.7	12	11	10	5	5	......	.....	......	100	75.0
15.0	12	12	10	4	3	3	.....	......	100	110.0
18.0	12	12	12	4	2	4	1	2	91	124.3
21.0	12	12	9	2	4	3	.....	......	100	122.1
24.0	12	12	10	3	5	2	.....	......	100	105.0
26.5	12	11	11	4	3	3	1	1	99	99.7
29.0	12	12	12	6	1	1	.....	4	66	49.9

Twenty-six days after planting, lesions were found on plants throughout the entire temperature range. Lesions which occurred at the extreme temperatures,[7] however, were very slight and in many cases consisted of little more than distinct browning of the outer tissues. The most severe destruction of the stem tissues occurred at the temperatures from 15° to 24° C. As in Experiment 1, few plumules or growing points were destroyed. The control plants were again found free from lesions.

TABLE IV.—*Average of results from pea Experiments 1 and 2, including dry weight of plants grown as controls in Experiment 3 (see figure 2)*

Average temperature.	Average percentage of plants injured.	Average total intensity of injury.	Average dry weight per plant.[a]
° C			Gm.
9.2	44.6	25.8	0.062
12.0	81.3	75.0	.112
15.0	83.3	105.0	.179
18.1	89.5	136.6	.222
21.0	70.0	101.0	.243
24.0	65.0	71.0	.235
26.6	45.0	50.0	.226
29.0	33.0	24.9	.216

[a] The figures represent the average of 12 plants on which determinations were made. The exaggerated severity of the damage occurring in the sterilized soil (Experiment 3) justified the inclusion of data on growth of the host plant with the more normal results of Experiments 1 and 2. (See Experiment 3.)

EXPERIMENT 3.—Greenhouse soil used in this experiment had been sterilized for two hours at 15 pounds' pressure and afterwards allowed to stand exposed to the atmosphere for three days before being placed in the cans. A small quantity of agar culture of *Corticium vagum* was then thoroughly mixed with the first 4 inches of earth in each can, and the seeds planted immediately thereafter at a depth of 2 inches.

As in the previous experiments, one can at each temperature was used for the growth of plants in the inoculated soil and a similar series was used as a control. After 16 days' growth, the plants in both series were photographed (Pl. 1). The control plants were then washed free from soil and the average dry weight of the plants determined for each temperature (Table IV and fig. 2). Observations on the diseased plants taken at the time are recorded in Table V and are shown graphically in figure 3.

TABLE V.—*Effects of growing peas at various temperatures in steam-sterilized soil infested with Corticium vagum (Experiment 3)*

Temperature at depth of 1 inch.	Number of seeds planted.	Number of plants grown in uninoculated soil.	Plants grown in inoculated soil.						
			Total number.	Number slightly injured.	Number severely injured.	Number cut off.	Number uninjured.	Percentage injured.	Intensity of injury (points).
° C.									
9.0	20	18	15	....	....	14	1	93.3	138.3
12.0	20	20	14	....	1	11	2	85.7	114.2
15.2	20	20	14	....	....	14	....	100.0	150.0
18.0	20	20	15	....	....	15	....	100.0	150.0
20.6	20	18	13	....	2	8	3	77.0	107.6
24.0	20	19	....	....	....	....	....	....	....
26.4	20	18	14	1	3	3	6	57.1	64.0
28.0	20	20	16	2	2	3	9	43.7	50.7

[7] The number of plants showing slight injury justified separating the group *slightly injured* into two classes—*very slightly injured* and *slightly injured*. Each per cent in the former class was allowed one-half point, while the latter class was allowed its former rating of one point (see page 432, paragraph 2).

Under these severe conditions total destruction of the plumule resulted at temperatures of 15° and 18° C. At 9°, while most of the seed germinated normally, only 1 plant out of a possible 29 escaped injury. At the higher temperatures the severity of attack decreased more rapidly than at temperatures below those most favorable for tissue destruction. Severe injury, nevertheless, was obtained at 28°. An interesting feature of the experiments appeared in the fact that damage to the primary roots occurred at temperatures of from 15° to 21°, although no visible lesions were found on the smaller fibrous roots of the plant.

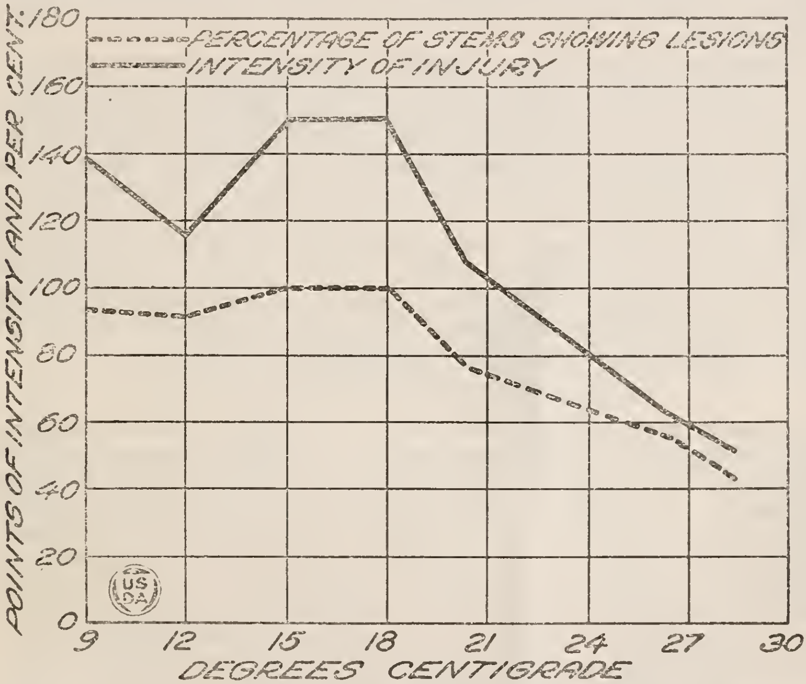

FIG. 3.—Effect of soil temperature upon the severity of injury caused to the pea by *Corticium vagum*.

DISCUSSION OF RESULTS OBTAINED WITH THE PEA

Results obtained from the three experiments on the pea agree essentially with those obtained by Jones in showing that within the temperature limits employed (9° to 29° C.) neither the high nor the low temperature entirely inhibited injury from *Corticium vagum*. However, in unsterilized soil, as in Experiments 1 and 2, the fungus is shown to be definitely limited in its pathogenic power at both the high and low temperatures. In these two experiments severe damage to the young plants was confined largely to temperatures of from 15° to 24°, with an optimum of approximately 18° C. Such favorable conditions for the pathogenicity of the fungus produced by sterilized soil, on the other hand, result in a much wider temperature range for the vigorous action of the fungus, although even under these conditions the most severe tissue destruction ranged between 12° and 24°. An equally definite optimum under these conditions was found at 18°.

In the earlier work on the potato (*13*), injury to the growing point of the young potato shoots appeared as a definite function of the rate at

which the young sprouts pushed through the soil in response to the temperature of the soil. With the pea, in the unsterilized soil of Experiments 1 and 2, the plumules were destroyed only at temperatures approximating the apparent optimum for tissue destruction (15° to 21° C.). In Experiment 3, however, under conditions more favorable for the pathogenic action of the fungus, growing-point injury occurred at 28°. Nevertheless, even under these severe conditions such injury was limited primarily to temperatures below 20°. As in the case of the potato, it appears quite probable that the rapid growth after germination played an important part in the protection of the plumules at the higher temperatures.

Normal pea growth was obtained throughout the entire range of temperatures of from 9° to 29° C. (Pl. 1, A). However, the most rapid germination and early growth, indicated by the time at which the plants appeared through the soil, occurred at 28° and 29°.[8] These results agree essentially with the optimum secured by Leitch (*10*). Dry-weight determinations showed the greatest growth of the normal plant for a period of sixteen days at 21° C. (Fig. 2 and Table IV.) Were the dry weights taken as an index to the temperature values at this period of the plant growth, it is clearly evident that the optimum for germination and early growth and the optimum for the later development of the plant would be widely different. This same relation was shown to exist for the potato under similar experimental conditions (*13*).

TEMPERATURE STUDIES WITH THE BEAN

Studies with the pea and the potato show clearly a similar soil temperature range for the pathogenic action of *Corticium vagum* on these two hosts. With both these plants 18° C. appeared optimum for tissue destruction. It will be recalled further that 18° approximates closely the soil temperatures found most favorable for the later and continued growth of both the pea and the potato. Whether this temperature relation between parasitism and host development is merely coincident with the two plants, due possibly to their similar temperature requirements for growth, or whether it is a condition determined primarily by a fixed character of the pathogen, does not appear clearly from the data obtained. Such data as Jones has supplied on cotton (Table I, fig. 1) support the latter possibility. However, to settle this question, additional experiments with other plants having temperature requirements different from either the pea or the potato appeared necessary. The work and observations of Reynolds (*12*), Reddick (*11*), Barrus (*4*), and Burkholder (*6*) on the relation of temperature to the growth of the bean suggested this plant as a favorable host for this additional study. Two experiments were accordingly made with this host.

Experiment 1.—Seven temperatures were employed, as indicated in Table VI. The soil used had been steam-sterilized and then inoculated with *Corticium vagum*[9] four weeks prior to the planting of the beans, and had grown during this period two crops of cress and radish seedlings. Before the beans were planted, however, the inoculated soil in the various cans was emptied and thoroughly mixed. Three cans were then filled with the inoculated soil at each temperature, and fifteen seeds[10] planted

[8] These temperatures were the highest which were maintained in the experiments and possibly do not indicate the optimum for early growth.

[9] The fungus used in these experiments was the same as that employed in Experiments 1 and 2 on the pea.

[10] The bean seed was grown at the Utah Agricultural Experiment Station and exhibited no signs of disease. Precautions were taken before planting to free the seed of all adhering organisms by treating for 10 minutes in mercuric chlorid solution (1:1,000).

2 inches deep in each can. Uninoculated soil previously used to check cress and radish experiments was similarly mixed and used for growing control bean plants.

Plantings were made on April 30 and the temperatures immediately adjusted. Seventeen days after the planting, on May 16, photographs were taken of the control plants and of one series of plants grown in the inoculated soil (Pl. 2, A and B). The data taken at this same date on plants grown in inoculated soil are shown graphically in figure 4 and in Table VI.

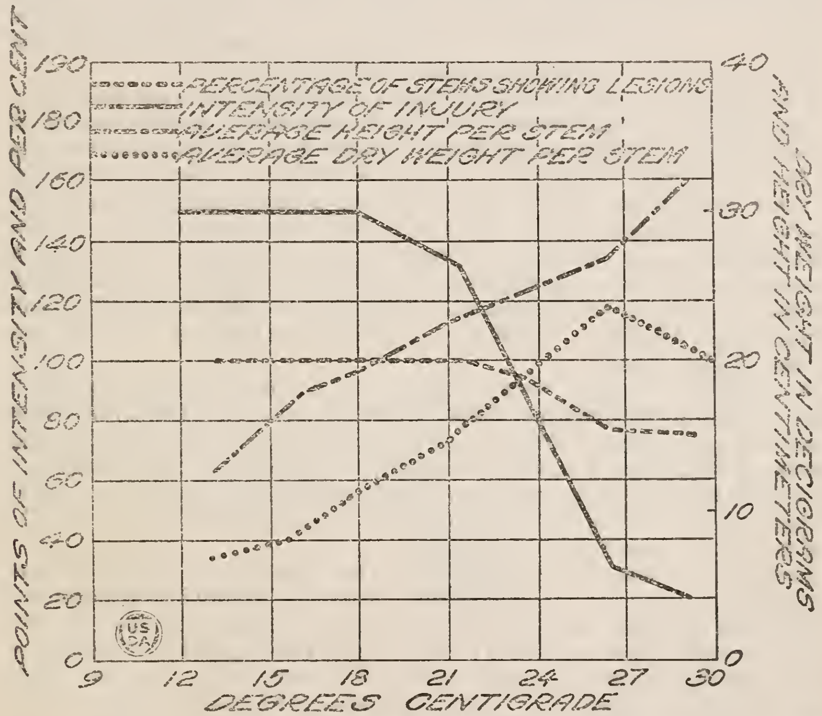

FIG. 4.—Effect of soil temperature on the pathogenic action of *Corticium vagum* on the bean. No plant survived at temperatures below 21° C. (see Pl. 2). The growth curves show the effects of soil temperatures upon host development, judged both from dry weight and height of plants.

TABLE VI.—*Effects of soil temperature upon the pathogenic action of Corticium vagum on the bean and also upon the development of the bean plant* (*Experiment 1*)

Temperature at depth of 1 inch.	Number of seeds planted.	Number of seeds grown in uninoculated soil.	Plants grown in inoculated soil.									
			Total number.	Number very slightly injured.	Number slightly injured.	Number severely injured.	Number cut off.	Number uninjured.	Percentage uninjured.	Intensity of injury.	Average height of plant.	Average dry weight of healthy plants 16 days old.
°C.											Cm.	Gm.
13.2	45	14	25	Plumule.	Destroyed.	......	......	......	100.0	300.0	12.19	0.073
16.0	45	15	31	"	"	......	......	......	100.0	300.0	17.78	.087
18.5	45	13	29	"	"	......	......	......	100.0	300.0	18.79	.118
21.5	45	13	36	......	4	5	27	......	100.0	263.7	22.8	.150
23.5	45	13	38	1	13	4	18	2	94.7	188.3	24.1	.186
26.5	45	15	42	23	5	3	1	10	76.2	60.8	27.0	.235
29.3	45	15	43	30	2	......	......	11	74.4	40.0	32.0	.313

Lesions occurred on most of the plants throughout the entire temperature range. However, at 29° C. only two plants showed any definite tissue destruction. Such lesions as were observed on the remaining twenty plants at this temperature consisted of but slight browning of the outer stem tissue. The degree of damage was much increased at 27°, and at 24° a severe decrease in crop resulted. Though not expressed in the table or the curves, the maximum degree of injury occurred, without doubt, at temperatures of 18° and 21°. At these temperatures the plumules of the embryos were entirely destroyed and the hypocotyl of the seedlings were in many cases decayed. Although no plants appeared above the surface of the soil at 13° and 15°, the tissue destruction of the hypocotyl, which had made considerable growth at these temperatures, was not as great as at 18° and 21°.

Experiment 2.—As is evident from results presented, the conditions under which the plants were grown in Experiment 1 were so severe as to allow of no accurate quantitative expression of the degree of damage in the soil at and below 21° C. In an attempt to reduce the severity of these conditions, and in order to secure a more definite idea of the effects of the fungus on the bean at these lower temperatures, the inoculated and uninoculated soil used for Experiment 1 was emptied into separate piles, each diluted with twice its weight of unsterilized garden soil, and separately mixed. Sixteen cans were then refilled and temperatures adjusted. Fifteen seeds were again planted 2 inches deep in each of the 16 cans.

At the time the data were taken, 16 days after planting, all plants were above ground, except those grown at 9.4° C. However, these were well germinated. At 15° and 18° the plants in the inoculated soil appeared somewhat irregular in height, but otherwise they could not be distinguished from those grown in the uninoculated soil. The results of the examination of the underground parts are recorded in Table VII and are shown graphically in figure 5.

Under these less severe conditions, the damage to the stems was limited to the soil temperatures below 26.5° C. Severe injury occurred only in the soil held at 12°, 15°, 18°, and 21°, while the plumule destruction was noted only at 12° and 15°. The highest percentage of stems showing lesions, together with the most severe type of tissue destruction, was found at 18°, although because of injury to the growing points, a greater total intensity of injury is shown at 15°.

Table VII.—*Effects of soil temperature on the pathogenic action of Corticium vagum on the bean (Experiment 2)*

Temperature at depth of 1 inch.	Number of seeds planted.	Number of plants grown in uninoculated soil.	Plants grown in inoculated soil.								
			Total number.	Number slightly injured.	Number severely injured.	Number cut off.	Number uninjured.	Percentage injured.	Intensity of injury.	Average dry weight of healthy plants.
°C.										Gm.
9.4	20	15	16	4			11	25.0	25.0	0.025
12.5	20	18	18	8	1	1	8	55.5	72.0	.066
15.0	20	17	15	6	1	3	5	66.6	113.2	.101
18.0	20	18	16	8	3		5	68.45	106.1	.131
21.0	20	18	18	5	1		12	33.3	38.7	.143
23.6	20	16	19	5			14	26.32	26.32	.190
26.5	20	19	19				19			.200
29.7	20	19	19				19			.206

DISCUSSION OF RESULTS OBTAINED WITH THE BEAN

In steam-sterilized soil *Corticium vagum* is shown to have a wide pathogenic range on the bean of from 9.4° to 29° C. With unsterilized soil, however, the fungus appeared definitely limited in its parasitic action under both the higher and lower temperatures tried in the experiments. In fact, under these more natural conditions, serious damage was confined entirely to the temperatures of 12.5°, 15°, 18°, and 21°, with a definite optimum for tissue destruction between 15° and 18. As with the pea and the potato, it is conceivable that the optimum might vary, contingent upon the conditions of the experiment, anywhere between 15° and 21°.

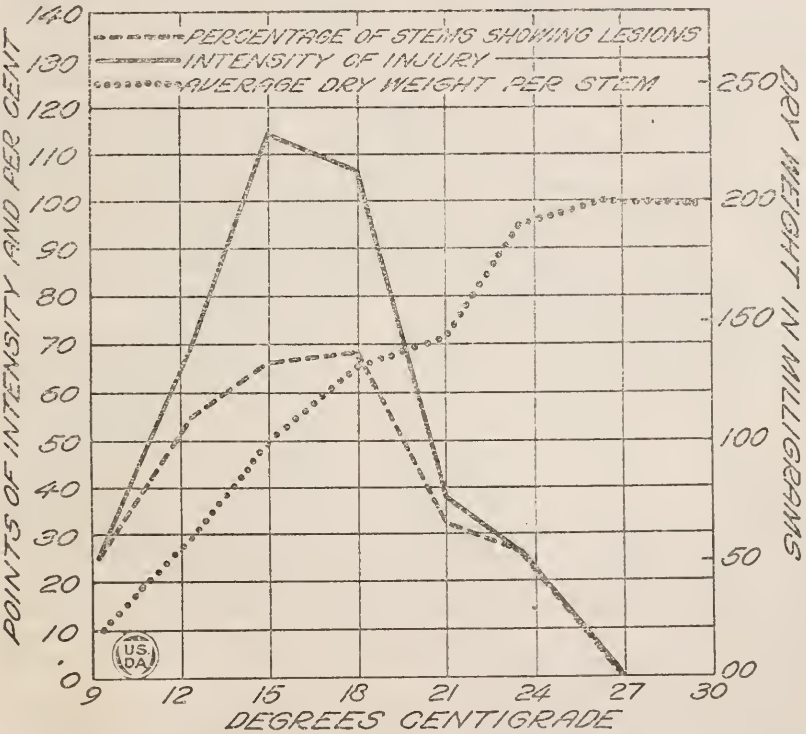

FIG. 5.—Effect of soil temperature upon the pathogenicity of *Corticium vagum* on the bean and upon the development of the host in uninoculated soil. The optimum temperature for the bean at the end of seventeen days lies clearly beyond the temperature range for the serious pathogenic action of the fungus.

General cultural experience shows the bean to be a plant which requires a high temperature for maximum growth and yield. Reddick (*11*) obtained the best growth at 28° C., while for germination Reynolds (*12*) found 30° to be the most favorable. Height measurements and dry-weight determinations in the present experiments, although not extensive, would indicate that the optimum soil temperature for the continued growth up to approximately three weeks, lies between 28° and 31°. The plants came up in all cases at the highest temperatures employed.

The parasitic relation of *Corticium vagum* on the bean is especially interesting in that the temperature most favorable for the growth of

the host falls completely outside of the dangerous pathogenic range of the parasite, and at such a high point as would allow the bean plants at their most favorable temperatures to escape practically uninjured. Jones' work (Table I, fig. 1) indicates that this same temperature relation holds equally true for the cotton plant. This agrees also with the field observations of Balls (*2*).

TEMPERATURE STUDIES WITH THE FUNGUS

Temperature requirements for the growth of *Corticium vagum* in pure culture have been determined with considerable definiteness by various workers. Rolfs (*15*) found 75° F. (24° C.) to be the most favorable for growth of the fungus. Hartman[11] obtained "no growth at 2°, slight growth at 8°, fair growth at 13° to 18°, profuse growth at 24° to 25°, fair growth at 30°, and no growth at 37° C." The fungus is shown by him to have a growth range of from 4° to 32° with an optimum at 24° to 25°. Balls (*3*) gives 5° to 32° as the growth range for the fungus and 23° as an optimum for continued growth. At 32°, he states, the fungus grows rapidly for two hours and then suddenly stops growth altogether. Balls (*3*) further reports a thermal death point of 50° for the young sclerotial cells when exposed at this temperature for a period of two minutes. He shows also that the optimum temperature for the growth of the fungus is a variable factor, definitely dependent upon the physiological history of the fungus as well as upon the immediate conditions under which the fungus is growing.

The wide temperature range through which *Corticium vagum* was found to destroy living plant tissue made it desirable to determine more accurately the temperature limits of the vital activities of the fungus, and to discover if possible its optimum temperature requirements for growth under varying conditions in pure culture. This latter relation is especially desired in view of the the low optimum of 18° C. found by the writer for the pathogenic activities of the parasite on the various hosts. The pressure of other duties, up to the present, has limited the work to the ordinary petri-dish method of study which, owing to the rapid growth of the mycelium, so shortens the period of observation as to lessen seriously the value of the results in interpreting the temperature reactions of the fungus. The data so far obtained, however, are of sufficient importance in their relation to the general problem to justify including them at this point.

In these studies small squares of a rapidly growing culture of the fungus, grown at 25° C., were placed in the center of the medium in each of the desired number of petri dishes. All the plates were then wrapped in sterile paper and kept at exactly the same temperature for 12 hours. At the end of this time the colonies were measured and the cultures placed directly in the incubators, which were held at the desired temperatures. Observations and measurements were made at regular intervals of 24 hours until the fungus had overgrown the media in the petri dishes. This, at the most favorable temperatures, occurred in about four days from the time that the temperatures were adjusted. Three series of the triplicate cultures were run at different temperatures. The average rates of growth obtained in series 1 and 2 are given in Table VIII and are shown graphically in figure 6.

[11] HARTMAN, R. E. A POTATO DISEASE CAUSED BY RHIZOCTONIA. A thesis submitted for the degree of master of science. Unpublished, typewritten copy on file in the University of Wisconsin library. 1915-16.

The results thus obtained agree essentially with those of previous workers. Growth occurred through a range of temperatures of from 4.6° to 32.5° C., with an optimum both for linear and aerial mycelial growth for 96 hours of between 25° and 27°. It will be noted from the data tabulated, however, that at the higher temperatures the initial growth rate was not maintained; a progressive decrease with time was noted at all the different temperatures between 23.6° and 32.5°, inclusive, and only at the temperature of 22.4° was the rate of growth for the first 24 hours maintained for the entire period of observation.

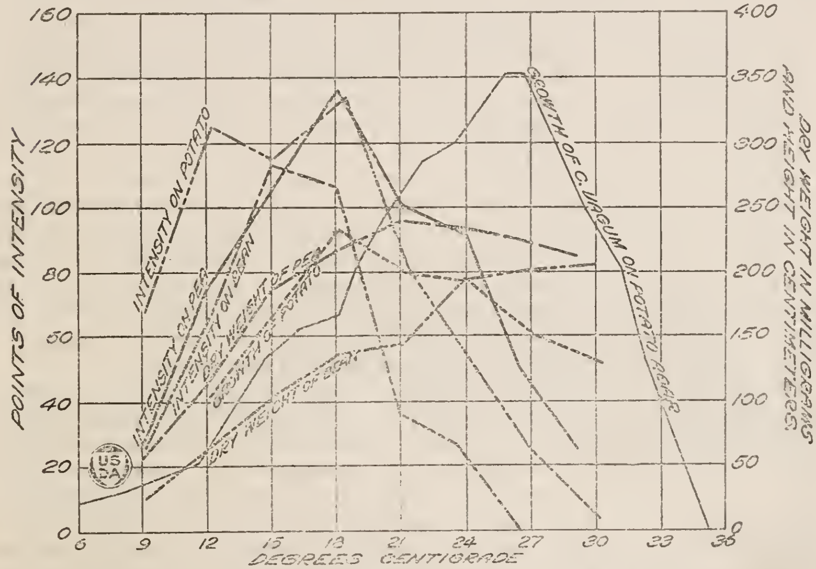

FIG. 6.—Showing the temperature range for the pathogenic action of *Corticium vagum* on the three hosts—pea, bean, and potato—in comparison with the temperature requirement for the growth of these three host plants (*13, tables on p. 465–472 and 475*) and for the growth of the fungus in pure culture.

TABLE VIII.—*Effect of temperature upon the growth of Corticium vagum on potato agar*

	Increased growth in diameter of colony for consecutive periods of 24 hours at different temperatures (°C).															
	4.6	8.2	10.9	14.6	16.3	18.1	19	20.1	22.4	23.6	25.7	26.6	29.8	31.2	32.6	35
Age of colony (hours):	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*
24	0.0	0.0	3.0	13.5	16.0	17.0	18.0	22.5	28.5	33.0	38.0	39.0	36.0	24.0	20.0	0
48	3.0	3.0	9.5	16.5	18.0	18.0	22.0	25.0	31.0	29.0	37.0	38.0	26.0	22.0	15.0	0
72	1.5	3.5	6.0	7.5	16.0	13.5	22.0	25.0	25.0	29.0	36.0	34.0	23.0	19.0	10.0	0
96	1.0	4.5	5.0	15.0	12.5	....	20.0	24.0	29.0	29.0	30.0	29.0	15.0	16.0	7.0	0
Average increase in diameter of colony	1.5	2.7	5.6	13.0	15.6	16.3	20.5	24.1	28.3	30.0	35.2	35.2	25.0	20.2	13.1	0
Average linear growth of mycelium	.75	1.35	2.87	6.5	7.8	8.1	10.25	12.1	14.1	15.0	17.6	17.6	12.5	10.1	6.55	0

This decided falling off in the rate of growth with time at the higher temperatures is especially significant, in view of the low temperature of 23° C. which was found by Balls (*3*) to be optimum for continued growth of the fungus. This would indicate that had the experiment continued, a much lower optimum temperature than that found for the 96 hours

would have been shown. In fact, a study of the growth reactions of the fungus at temperatures of 23.6°, 25.7°, and 26.7° would suggest a possible optimum of 23.6° at the end of 120 hours. Balls noted the effect of the time element in his studies on the sterile stage of *Corticium vagum*, or "sore-shin" fungus, and studied the phenomenon in detail. He showed that the stopping point for the apical growth of the hyphae was lowered with increase of time. Again, this author points out that the "time element" varies with the different media on which the fungus is grown. On hard agar, he states, the growth was considerably inhibited by high temperature; on soft agar at the higher temperatures, inhibition of rate of growth became evident with increase of time; while in liquid media this slowing up of the rate of growth was delayed, the delay being greater with increase of volume. From considerable experimental evidence, Balls (*3*) concludes that this inhibitive effect of high temperature is occasioned entirely by the by-products from the metabolic process which accumulate at these temperatures, both within and without the cell, to such an extent that growth is limited by them, and he further suggests that the accumulation of such substances may play an important part in limiting the pathogenic power of the fungus at these higher temperatures. It is interesting to note that a similar decreased rate of growth with increase of time was found by Lehenbauer (*9*) to be an important factor in the growth of the corn plant at the temperature of 32° C. and above. The observations of this writer, however, were not continued over a sufficient period to give definite data on this point.

From a study of the data in Table VIII, it is clear that the temperatures shown to be most favorable for the growth of the fungus may be considered as optimum for a period of 96 hours only. Furthermore, as has been pointed out by Blackman (*5*), Lehenbauer (*9*), Leitch (*10*), and others, the optimum temperature value for growth under any specific set of conditions is quite without meaning unless the time limits for the period of the observations are accurately stated. Likewise, we are forced to conclude from these studies and from the work of Balls (*3*) that the actual optimum temperature for the growth of any strain of *Corticium vagum* will depend upon a large number of factors relating to the condition and history of the fungus, as well as to its specific environment.

TABLE IX.—*Ratio of increased temperature to rate of growth of the fungus Corticium vagum over a period of 96 hours*

Temperature range.	Comparative increase growth for each 10° C.	Coefficient.
°C.	Cm.	
4.6 to 14.6	0.76 to 6.5	8.50
8.2 to 18.1	1.35 to 8.1	6.00
10.9 to 20.1	2.87 to 12.1	4.20
14.6 to 23.1	6.50 to 15.0	2.30
16.3 to 25.3	7.80 to 17.5	2.24
18.1 to 29.8	8.10 to 12.5	1.54
19.8 to 29.8	10.25 to 12.5	1.21
20.1 to 31.2	12.10 to 10.0	0.82
23.6 to 32.6	15.00 to 6.5	0.43
25.3 to 35.0	17.50 to 0	0

The ratio at which the fungus growth increased with rise in the temperature is an especially interesting phase of the studies. Balls (*3*) found that the growth rate of the specific strain of the Rhizoctonia stage of *Corticium vagum* with which he worked accorded for a very short period with Van't Hoff's law. This same growth relation has been found true within certain limits for the pea by Leitch (*10*) and for the corn by Lehenbauer (*9*). In these studies a constant coefficient for each increment of 10° C. rise in temperature was not found; on the other hand, as shown in Table IX, a progressive decreasing coefficient resulted with increase in temperature. Owing in part to the operation of the possible "time factor" during the 96 hours of exposure, the specific value which might be predicted from the Van't Hoff law was approximated only within the narrow range between 14° and 25° C. What relation the progressively decreasing coefficient might have to the pathogenicity of the fungus is not clear, although it would appear significant in view of Balls's conclusions that "some (deleterious) products are formed at the low as at the high temperatures but at a much slower rate." Were such varying quantities of by-products present in the mycelium at the different temperatures as suggested, it would appear that they might be important in determining the varying degrees of closeness to which the hyphae of *Corticium vagum* grew to the substratum throughout the temperature range for fungal growth, a relation described in the following paragraph. As closeness of contact is of undoubted importance in the pathogenicity of the fungus, the decreasing ratio value might also have special significance as a factor in its pathogenic relation and be definitely indicative of greater parasitic possibilities on the part of the fungus at the lower temperatures where, owing to slow accumulation or absence of poisonous products, the hyphae adhere, and even embed themselves, in the substratum.

In addition to the fact that the hyphae grew exclusively from an apical zone, Balls (*3*) determined that the mycelia exhibited different growth characteristics at various temperatures. In liquid media at 20° C. he found that the hyphae grew straight and became slightly branched. At 34° in liquid media a fluffy mycelial growth resulted; more numerous short hyphae and fewer resting cells developed than at 20°. A number of these features were especially evident in the present study. At the lower temperatures the hyphae became sparsely branched and grew widely separated in the colony. The most characteristic feature lay in the fact that the hyphae at temperatures below 15° grew closely in contact with the medium (agar), and at still lower temperatures, as stated, definitely embedded themselves in it. Mycelia so developed appeared hyalin in color, and for considerable periods would remain almost indistinguishable from the media. Normal pigmentation and resting-cell formation were found to be greatly delayed at these lower temperatures as compared with the higher temperatures. With higher temperatures the mycelia branched more profusely and produced a more superficial type of growth upon the substratum. Between 24° and 28° profuse mycelial growth resulted frequently with definite aerial hyphae, often growing in a direction at right angles to the substratum. The characteristic brown pigment, together with the resting cells and consequent sclerotia formation, appeared at its maximum within this particular temperature range. These latter characteristics were found to decrease with increase of temperature above 27° and 28°, resulting in smaller colonies consisting of fluffy, aerial, short, but frequently branched, hyphae.

GENERAL CONSIDERATIONS

The uniform parasitic behavior of *Corticium vagum* on its several hosts in relation to the temperature of the soil is of special interest. Figure 6 shows the temperature range for the pathogenicity of *Corticium vagum* on the three hosts—pea, bean, and potato—in comparison with the temperature requirements for the growth of these plants.[12] It is evident that the optimum temperature for the growth of the bean, as undoubtedly as for the cotton (see Table I and fig. 1), is entirely outside the temperature range for dangerous pathogenic action of the fungus, and at such temperatures as would permit these plants to grow at their best, practically uninjured. On the other hand, the optimum for the continued growth of the pea and for the potato (18° C.) lies well within the range and approximates closely the temperature found most favorable for the pathogenic activity of the fungus. It would appear evident from the data accumulated, that this close approximation in the case of the pea and the potato is merely coincident, both the hosts in question being cool-temperature plants with similar temperature requirements. The fact that its optimum temperature for pathogenicity remains the same for the cotton and bean as for the pea and the potato would indicate that the temperature requirements for the parasitic relations of *Corticium vagum* and its various hosts is not influenced seriously by the species of hosts attacked, nor by the temperature requirements of any host, but is without doubt a condition determined primarily by a fixed physiological characteristic of the pathogen. It is further suggested that in general the resistance of these hosts as affected by the temperature of the soil plays a minor part in influencing the pathogenic state.

It is significant that the temperature range so far as determined for the parasitic activities of *Corticium vagum* agrees essentially with that found for its growth as a saprophyte in pure culture. While slight growth in pure culture occurs as low as 4.6° C., the severe damage which was found on the potato stems at 9° (*13*), the lowest temperature maintained, indicates that the fungus may become pathogenic under specially favorable conditions at temperatures considerably lower than 9° and probably as low as the minimum found for hyphal growth. Further, it should be recalled, in view of the relative low maximum (32.6°) for the growth of the fungus in pure culture, that under severe conditions, such as obtained for the pea and the bean in steam-sterilized soil, the highest temperature (29.5°) tried in the experiment did not entirely inhibit tissue destruction. It is important to note in this relation that a considerable difference appears between the optimum temperatures for pathogenic action and for hyphal growth (fig. 6). As previously indicated, however, we have no accurate data at present as to the possible optimum for growth of the fungus under the different conditions in the soil. It appears definite that the exact optimum for the saprophytic growth will vary within a considerable range, dependent upon a number of factors. Results obtained by Balls, together with the decreasing rate of growth noted at temperatures above 22.4°, suggest a possible optimum for saprophytic activity in the soil not far different from the low optimum found for its parasitic activity.

There appears no explanation of the fact, however, that the optimum temperature for tissue destruction on the various hosts falls considerably below that found most favorable for mycelial growth in pure culture.

[12] Temperature data for the potato in figure 6 are obtained from the earlier publication of this series (*13*). The reader is referred to this article for a full discussion of this relation.

Balls points out that the sensitiveness of *Corticium vagum* to its own metabolic products would explain the decreased pathogenicity at the higher temperatures. Again, a decrease in pathogenic power may result from the definite tendency of the fungus to grow more superficially on the substratum at the higher temperatures, while closely adhering to the substratum at the lower temperatures. With respect to the optimum temperature for tissue destruction, it is quite probable that in the last analysis we are dealing with an enzym, or a group of enzyms, which are secreted more abundantly at 18° C., or which react more vigorously on the host tissue at this particular temperature. At present we have no direct evidence of such enzym activity of *Corticium vagum.* It is interesting in this connection, however, to note that Jones (*8*) obtained a more abundant secretion of cytolytic enzyms from *Bacillus carotovorus* at 18° to 21° than at the higher temperatures of from 25° to 28°. Studies of the enzymatic activities of *Corticium vagum* would undoubtedly throw much light on this particular relation.

The nature of the parasitism exhibited by *Corticium vagum* offers interesting material for speculation. In the first place, it is evident that in the injury and destruction of growing points of the potatoes and plumules of the pea and the bean, we are dealing largely with a question of escape. As has been suggested, the early growth of the various hosts in the soil above 21° C. is so rapid as to permit the tender bud portion to push through the soil with little or no injury. This was found especially true of the potato shoots. On the other hand, the lower temperatures retard the host growth and increase the exposure at just those temperatures which are most favorable for tissue destruction. Such retardation as shown is frequently disastrous. With tissue destruction in general, however, it appears that we are concerned with a specific type of parasitism determined, as stated, by a fixed character of the fungus and one closely allied to its saprophytic activity. What this fixed physiological factor, or factors, in the fungus is remains undetermined. Favorable soil temperature and closeness of contact, however, appear as important relations in this peculiar parasitism.

It seems reasonable to suggest that, given an opportunity for closeness of contact, the action of the fungus in the soil upon living tissue is not widely different from that on nonliving organic matter. Were such the case, it appears possible that in the pathogenic activities of *Corticium vagum* we are dealing with the action of one or a group of enzyms whose temperature relations differ but little, regardless of whether the substances acted upon under natural conditions of the soil belong to the living or nonliving organic world. The approximate coincidence of the temperature range for saprophytic growth and for parasitism, the apparent complete lack of host specialization, the similar temperature range for destruction of tissue of the various hosts, together with the peculiar nature of the lesions produced by the fungus, and finally the wide range of hosts, limited in number perhaps only by our lack of observation—all seem to support such a view. With *Corticium vagum* it appears that we are concerned with a peculiar type of parasitism quite distinct from that exhibited by the more specialized parasites. The recent works of Ames (*1*), Edson and Shapovalov (7), and Shapovalov (*16*), are interesting in the suggestion that a large number of saprophytes may destroy living tissues under certain conditions especially favorable for their growth.

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SUMMARY

(1) Studies on the pathogenicity of *Corticium vagum* show that the fungus may become a vigorous parasite on the underground parts of both the pea and bean. The severity of damage resulting from the attack of this fungus on the two plants is shown definitely to be conditioned by the temperature of the soil.

(2) *Corticium vagum* may produce lesions on the pea through a soil temperature range of from 9° to 29° C. The greatest damage is found to result between 12° and 26°, with a definite optimum for tissue destruction at 18°. Essentially these same temperature relations are found for the pathogenic action of the fungus on the bean. Within the limits tried (9° to 29.5°) neither the high nor the low temperatures entirely inhibited injury from the fungus on bean stems, although, in unsterilized soil, damage to the bean was found to be limited to temperatures of 12.5°, 15°, 18°, and 21°, with a maximum amount of injury at 15° and 18°. Plumule destruction on the two plants, except under exaggerated conditions, occurred only at temperatures below 21°. These temperature values are practically the same as those found for the pathogenic action of *Corticium vagum* on the potato.

(3) Sterilized soil inoculated with a pure culture of *Corticium vagum* increased greatly the amount of injury caused by the fungus on both the pea and the bean. This is at variance with the results obtained in the studies of the potato.

(4) The cardinal temperature for the pathogenicity of *Corticium vagum* remained the same for the various hosts—potato, pea, bean, and cotton. Lesions occurred on all these plants from 9° to 29° C., with a general optimum for tissue destruction near 18°. The growing-point and plumule destruction on the various plants was exhibited at practically the same range of soil temperatures for all hosts studied. These various temperature relations held true for all the different strains of the fungus used in the experiment.

(5) The temperature requirements for the pathogenic action of *Corticium vagum* on its various hosts appear definitely as a fixed inheritable characteristic of the fungus, more or less independent of the temperature relations of the host on which it becomes parasitic.

(6) *Corticium vagum* grows in pure culture from 4.6° to 32.6° C. with an optimum for a period of 96 hours between 25° and 27°. The growth rate of the mycelium on hard agar between 23.6° and 32.6° decreases with the time of exposure. This fact suggests a much lower optimum for the continued growth of the fungus. At the lower temperatures hyphae embed themselves in the artificial substratum and retain their hyalin color and active state much longer than at higher temperatures. At 20° to 30° the mycelial growth is much more superficial, producing aerial hyphae which grow frequently at right angles to the surface of the medium. This closeness of growth in contact with the substratum at the lower temperatures probably plays an important part in the ability of the fungus to attack living tissues at temperatures below 21°.

(7) The temperature range indicated by the minimum and maximum temperatures for the pathogenicity of *Corticium vagum* approximates closely the temperature range found for its saprophytic activities. The optima for these physiological processes, however, vary widely. The temperature optimum for mycelial growth of the fungus, in fact, appears

to bear no direct relation to the temperature requirements for maximum pathogenicity. The ratio of the rate of growth with increase of temperature conforms to Van't Hoff's law only between the temperatures of 14° to 24° C.

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PLATE 1

A.—Peas grown in uninoculated soil at the various temperatures of 28°, 26.6°, 24°, 20.6°, 18°, 15.2°, 12°, and 9° C. Twenty seeds were planted in each pot. Plants remained free from lesions.

B.—Peas which survived in steam-sterilized soil inoculated with *Corticium vagum* and held at the various temperatures as designated in A. Considerable damage occurred at 28.6° C.

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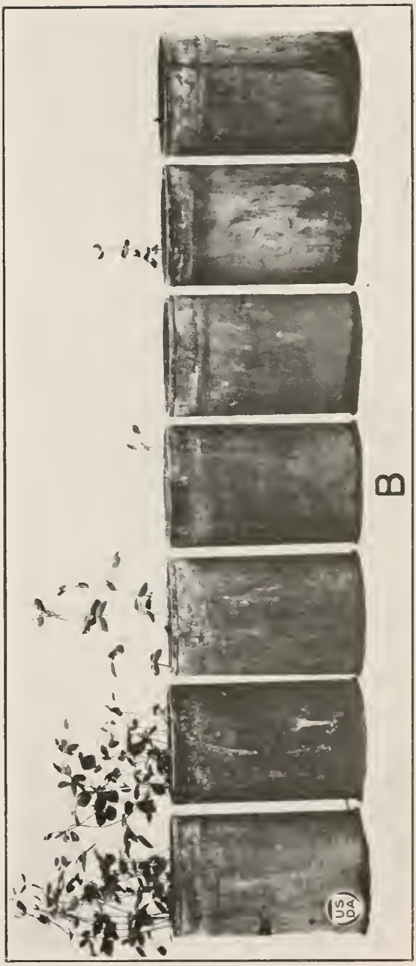
A



B

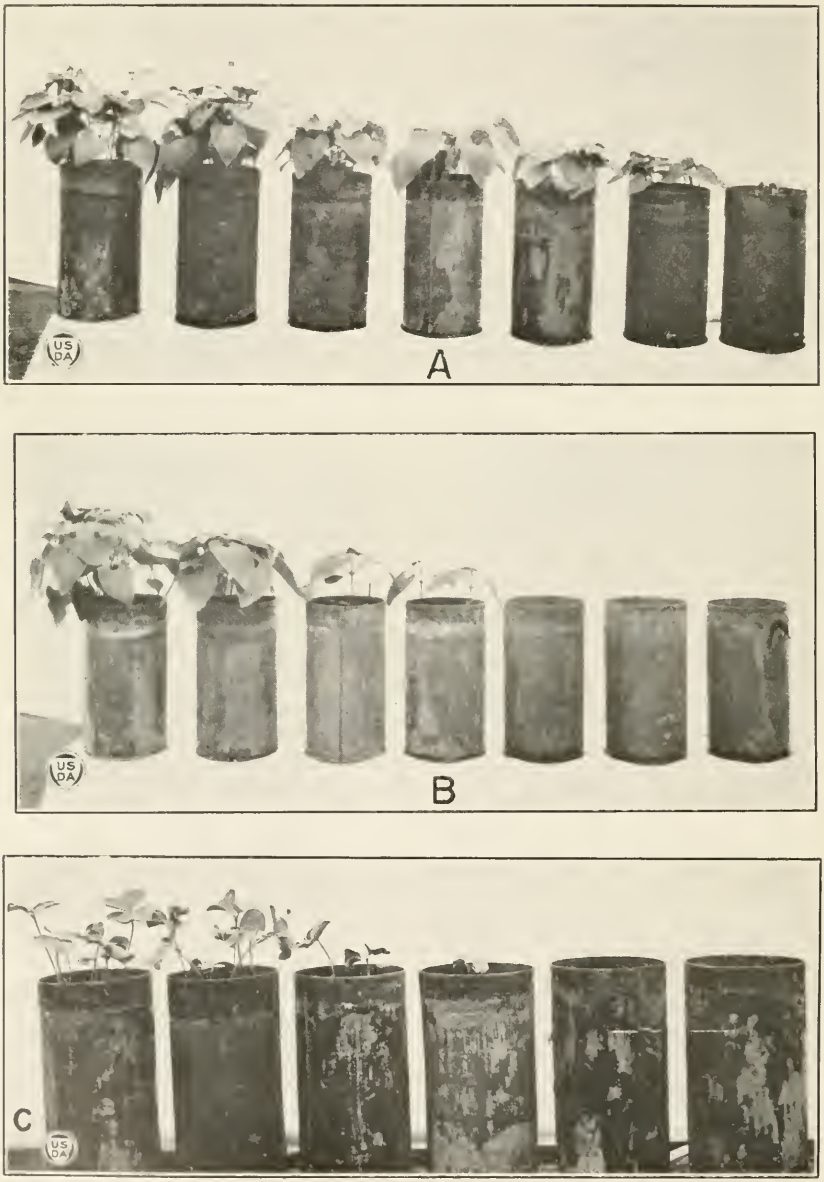

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PLATE 2

A.—Bean plants from Experiment 2 grown in uninoculated soil at temperatures of 29.3°, 26.6°, 23.5°, 21.5°, 18.5°, 16°, and 13.2° C. Germination and growth occurred throughout the entire range. Fifteen seeds were grown in each pot. Dry-weight determination and height of plants are recorded in Table VI.

B.—Beans grown in soil inoculated with *Corticium vagum* and held at the various temperatures as indicated in A. Very slight injury occurred at 29.3° C. (See Table VI.)

C.—Cotton plants which survived in infected soil at the various temperatures of 28°, 26°, 23.5° 18°, and 15° C. Seven seeds were planted in each pot. At the same temperatures in soil to which *Corticium vagum* had not been added the control plants grew perfectly, except that at 15° C. only half of the seeds germinated. (Photograph provided by F. R. Jones.)

GROWING EXPERIMENTAL CHICKENS IN CONFINEMENT[1]

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INTRODUCTION

This paper is an outgrowth of a research begun in 1916 by one of the authors (Ackert), who undertook a series of studies on the life histories of fowl cestodes. Early in the work it was realized that the raising of large numbers of chicks in confinement presented a problem in itself. Although the chicks made rapid gains during the first two months, they soon began to show signs of lameness, and in a few weeks became so weakened and abnormal as to jeopardize the experiments. On consulting the literature of the subject, it was found that other investigators had experienced somewhat similar difficulties.

Drummond [2], after a series of tests, concluded that a diet sufficient to produce normal development under range conditions was insufficient for chicks raised in the laboratory. By using a mixture of chicken feed, cabbage, and charcoal, and by replacing the water with milk, Funk [3] was able to reduce greatly the mortality, even though the chicks were kept in small cages. Osborne and Mendel [4] succeeded in raising to maturity some apparently normal fowls by supplying crude fiber in the form of paper pulp and blotting paper as a "ballast." Difficulties most nearly identical with those of the present writers were experienced in the rearing of chicks by Hart, Halpin, and Steenbock,[5] who state that "In the rearing of baby chicks in confinement a difficulty serious and obscure in its etiology is the one characterized by poultrymen as 'leg weakness.' This trouble usually develops in 4 to 6 weeks after hatching, but the writers have seen it show itself 4 to 6 weeks later. The principal symptoms shown by the bird are, first, an unsteady gait, developing into difficulty of locomotion with a tendency to remain squatted a good part of the time; a pronounced ruffled condition of the feathers; an anemic condition of the wattles and comb; and a swelling of the leg joints, which is sometimes permanent. A loss of appetite accompanies these conditions and usually death follows suddenly." These symptoms are very similar to those shown by the earlier lots of the writers' chicks, except that they retained a good appetite for grain and often lived for several weeks.

Hart, Halpin, and Steenbock, by incorporating 10 per cent of paper in the ration, succeeded in raising normal chicks in confinement for 18

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[2] Drummond, Jack Cecil. observations upon the growth of young chickens under laboratory conditions. *In* Biochem. Jour., v. 10, p. 77–88, 1 pl. 1916.

[3] Funk, Casimir. the study of certain dietary conditions bearing on the problem of growth in rats. *In* Jour. Biol. Chem., v. 27, p. 1–14, 4 fig. 1916.

[4] Osborne, Thomas B., and Mendel, Lafayette B. the growth of chickens in confinement *In* Jour. Biol. Chem., v. 33, p. 433–438, pl. 4–6. 1918.

[5] Hart, E. B., Halpin, J. G., and Steenbock, H. use of synthetic diets in the growth of baby chicks. a study of leg weakness in chickens. *In* Jour. Biol. Chem., v. 43, p. 421–442, 2 pl. 1920.

weeks, at the end of which time they were discarded. The method was so successful for those investigators that the present writers attempted to use it, but, owing to some unknown cause, the chicks discriminated against the feed containing the paper and failed to eat an amount sufficient to prevent leg weakness. After many trials, the method about to be described of raising chicks in confinement was developed, and, since it has been successful with rather large numbers of fowls, it is described with the hope that it may be of assistance to other experimenters. The writers wish to express their appreciation of the friendly cooperation of Dr. J. S. Hughes and Prof. L. F. Payne.

EQUIPMENT

The parasitology animal house in which the chicks were raised occupies a portion of the first floor of a two-story frame building. Screens on windows and door aid in excluding flies and other insects from the house. In the interior a hallway separates the sterilizing room, feed room, and laboratory on the north from the four rectangular pens on the south. Each pen, separated from its fellow by a screened partition, is 8 feet wide by 32 feet long, and extends north and south. Ample light and ventilation are afforded by large windows on all sides except the west. The house is steam-heated and is usually kept just warm enough to prevent freezing during the coldest weather. For small chicks, both kerosene and electrically heated hovers have been utilized, but at present a high-pressure steam radiator with a conical tin cover is placed in the partition between each pair of pens (Pl. 2, B, right).[6] Detachable canvas curtains aid in retaining the warm air in these hovers. For the older chickens, the roost and dropping board are placed along one side of the pen so that the fowls may select places out of the drafts. In the summer the screened partitions tend to increase the heat, but by spraying the pens with cold water the temperature is so reduced that the chicks suffer little from the heat.

CHICKENS

In the early experiments three breeds of chickens were used: Barred Plymouth Rocks, Buff Orpingtons, and Single Comb White Leghorns. The weight and comparative inactivity of the first two breeds seemed to make them more susceptible to leg weakness, for they showed abnormal symptoms sooner than did the alert Leghorns, though all were kept in the same quarters. After the first two years, therefore, only Single Comb White Leghorns were used.

All of the chickens were hatched in incubators, and placed at once in the pens. Lots of baby chicks have been secured from local poultrymen, the College Poultry Farm, and from commercial hatcheries.

As is well known, excellent care of baby chicks during the first two weeks is imperative. A temperature of about 100° F. is maintained in the hover. Sand, oyster shell, and clean water are kept constantly before them and a small amount of feed is given about every two hours. Shallow drinking fountains which exclude the chicks have the advantages of freedom from contamination and of keeping the chickens dry. As the chicks grow older, the board floor, 2 feet above the ground, is gradually covered with a litter of wheat straw. The fine straw and droppings are removed

[6] Photographs by F. E. Colburn.

each week and fresh straw added, so that the scattered grain disappears in the litter, thus making the chickens scratch for the feed. When the chicks attain the age of two to three weeks, dry mash is kept before them in a hopper, and containers of sand and oyster shell are also provided. Green feed and skim milk are given at least twice a week.

HISTORY OF THE LOTS OF CHICKS

During 1916–1918 the experimental chicks were grown in a small isolated house (Ackert)[7] with cement walls and floor, the latter being covered with sand. The baby chicks were started in this house only during the spring and summer months. When fed on rations of cereals, bone ash, and green alfalfa they thrived during the first eight or ten weeks; then lameness and other disorders began to develop. Since many of the parasitized chicks or their controls died in these crowded quarters before the termination of the experiments, the present animal house was provided.

The next year (1919) the experiments were conducted in the parasitology animal house already described. In these light, roomy quarters, with rations adapted from Lippincott,[8] good results were obtained. During the first week the baby chicks were given soft feed, such as bread or corn pone mixed with rolled oats and moistened with skim milk. The amount of rolled oats was gradually increased and such grains as wheat and cracked corn were added until the chicks were about two and a half or three months old, when they were put on a regular ration of hopper-fed dry mash, scratch grain in the litter, green alfalfa and skim milk. The dry mash consisted of five parts of shorts, five of bran, three of oil meal, and one of bone ash. The scratch grain was a mixture of two parts of cracked corn, two of wheat, two of kafir, and one of oats. Liberal amounts of fresh, green alfalfa, cut into half-inch lengths, were fed daily, and skim milk was before them practically all of the time. In the winter sprouted oats supplanted the green alfalfa.

In this lot of chicks only 3 died of leg weakness. The remaining ones (29 pullets and 2 cockerels) developed normally, averaging in weight 1,383.5 gm. at 1 year of age. One pullet began to lay when 5 months and 8 days old; and in 8 months, from November 14 until July 16, the lot laid 195 dozen eggs, although the egg production of 17 of the pullets was considerably reduced because from March 28 to May 28 they received no alfalfa and no skim milk. One of these hens is shown in Plate 2, A.

Fifteen chickens from this lot were kept in these pens for three years, and although not fed for heavy egg production, they laid well until they were discarded. At that time the ovaries of all but two contained well-developed eggs. One of the two had several partly reabsorbed eggs in its body cavity, while the other, which had a small ovary, had probably never laid many eggs. The largest hen (Pl. 1, B, left) weighed 3,288.6 gm., and the smallest (Pl. 1, B, right, front) 1,360.8 gm. The lot, a few of which are shown in this figure, averaged in weight 2,041.4 gm. The cock (Pl. 1, B, center), a vigorous bird, weighed 2,721.6 gm. The fertility of the eggs from these hens was high. A lot of 25 eggs incubated at the College Poultry Farm in February, 1920, were

[7] ACKERT, James E. ON THE LIFE CYCLE OF THE FOWL CESTODE, DAVAINEA CESTICILLUS (MOLIN). *In* Jour. Par., v. 5, p. 41–43, pl. 5. 1918.

[8] LIPPINCOTT, William Adams. POULTRY PRODUCTION. Ed. 2, rev. 517 p., 233 fig., 1 map. Philadelphia and New York. 1916.

all fertile, and yielded a hatch of 18 strong chicks. They were of average normal size, exceeding in weight an equal number of chicks taken at random from the College Poultry Farm. Their gains in weight likewise slightly exceeded those of the latter. These chicks (offspring of those grown in confinement) grew to maturity, laid at the usual age, and behaved normally in all respects. One of them is shown in Plate 1, A.

More rapid growth and generally better results were obtained in 1921 by beginning with buttermilk chick feed mixed with skim milk, and gradually changing to a ration of equal parts of wheat and corn chop as a scratch grain and a hopper-fed dry mash consisting of equal parts of corn meal, bran, shorts, beef scraps, and one-third part bone meal. This lot received green alfalfa after the first week and skim milk after the second. Although these chicks were in transit eight days, having their vitality greatly reduced, they averaged in weight 163.3 gm. in seven weeks and attained an average weight of 1,927.9 gm. at the age of 7 months. They laid their first eggs when 5 months and 22 days old, and continued to lay well until they were discarded in May, 1922. Plate 2, B, shows a group of these fowls shortly before they were discarded. Similarly, in May, 1922, 150 baby chicks shipped from Michigan were started on the buttermilk chick feed. From the first these chicks made gains which compared favorably with the average daily growth for chickens of this breed. Under conditions similar to those of the previous lots, 125 of these fowls reached maturity without any sign of leg weakness, and began to lay at the early age of 4 months and 27 days.

Some workers have experienced greater difficulties from leg weakness and other disorders with chicks hatched in late summer or fall than with those hatched earlier in the year. This has not always been true here. In the fall of 1921, 50 baby chicks were secured from Ohio. They received the same care as those hatched in the spring, and, although some were smothered, none of the survivors showed the slightest signs of leg weakness. They began to lay when 5 months and 7 days old, and the ovaries of all but one contained well-developed eggs when the chickens were discarded the following May.

However, in September, 1920, a lot of 50 chicks made good gains for about eight weeks, then they began to suffer from leg weakness. Soon all contracted it, and many died or were killed. Nine that recovered began laying at the age of 5 months and 4 days. Concerning the onset of the attack, the first abnormal symptoms appeared when the chicks were rapidly increasing their weight and plumage. Restlessness and perverted appetites appeared, the chicks showing an avidity for grain and an aversion for green feed and skim milk. The lameness and other characteristic symptoms soon followed. Whether or not the season of the year (fall) was effective in producing the abnormal appetite of these chicks is problematical, but the short, more or less cloudy days of late autumn are not conducive to activity for fowls raised in confinement.

DISCUSSION

Among the apparent factors of the production of abnormal chickens in the first experiments here were insufficient litter and crowding. In the course of two months these factors probably reduced the activity of the chicks, and paved the way for perverted appetites and leg weakness. With these difficulties obviated, and under the conditions described, several hundred chickens have been raised to maturity without develop-

ing leg weakness or any other apparent disorder. That they were normal seems evident from their thrifty appearance—their smooth feathers, clear eyes, and good color of comb and wattles. An average lot is shown in Plate 2, B. Their activities of scratching, dusting, brooding, crowing, and fighting, and their age of maturing, their weight, egg production, and fertility, all indicate that they were normal Single Comb White Leghorns. Moreover, 15 fowls from one lot thrived under these conditions for three years, when they were discarded.

On the other hand, the idea must not be entertained that here is an easy method of raising experimental chickens and that leg weakness has been banished. Unless there is ample light, ventilation, room and cleanliness, and unless the rations and care are administered consistently, especially during the first three months, discontent, inactivity, diminished or perverted appetites and leg weakness are almost certain to appear.

SUMMARY

(1) While experimenting in 1916 with chickens raised in confinement, a disorder known to poultrymen as leg weakness developed in the flock and interfered with the experiments.

(2) Single Comb White Leghorns proved to be less susceptible to the ailment than the heavier breeds, and were used for all subsequent experiments.

(3) Chicks hatched in incubators were placed at once in screened pens. Best results have come from the use of guaranteed chicks from commercial hatcheries.

(4) Marked improvement in the results of raising the chicks was accomplished in 1919 by the aid of well-lighted, screened pens, in a steam-heated frame building, and by the adoption of an adequate diet, consisting of common grains, a dry mash, green feed, skim milk, oyster shell, charcoal, and water.

(5) Light, roomy, well-ventilated pens with clean litter (preferably wheat straw) are valuable assests in keeping the chickens active and healthy.

(6) Under such conditions several hundred experimental chickens have been raised to maturity without showing any abnormal symptoms. Their growth, behavior, egg-production and weight have been similar to such performances of average Single Comb White Leghorns. From one lot, 15 individuals thrived in the pens three years, their eggs yielding normal hatches and their chicks developing regularly.

(7) The contention that chicks hatched in late summer or fall do not develop as well as spring chicks is partially supported, as leg weakness since 1919 has been confined to a lot of fall chicks. Reduced activity, possibly due to short, cloudy days, seems to lead to diminished or perverted appetites. On the other hand, no leg weakness or other disorder developed in a lot of chicks hatched in the fall of 1921.

(8) Failure to take liberal quantities of green feed and skim milk, either from lack of supply or loss of appetite, seems to induce leg weakness in two to three months' old chicks that are rapidly increasing their weight and plumage.

PLATE 1

A.—Year-old cock grown in confinement. Offspring of chickens kept one year in confinement.

B.—Chickens which have been kept in confinement three years.

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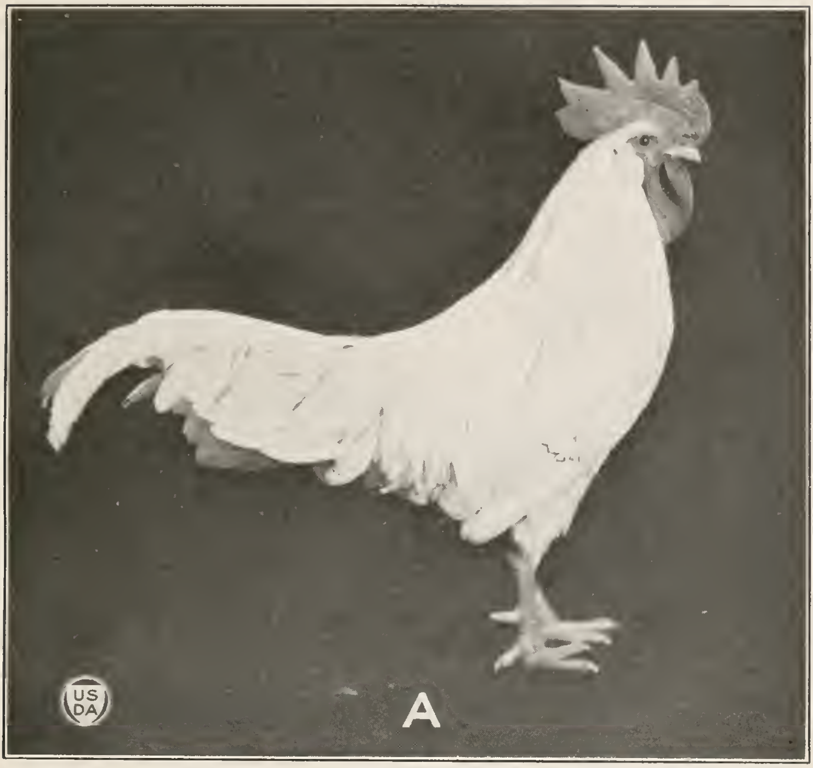

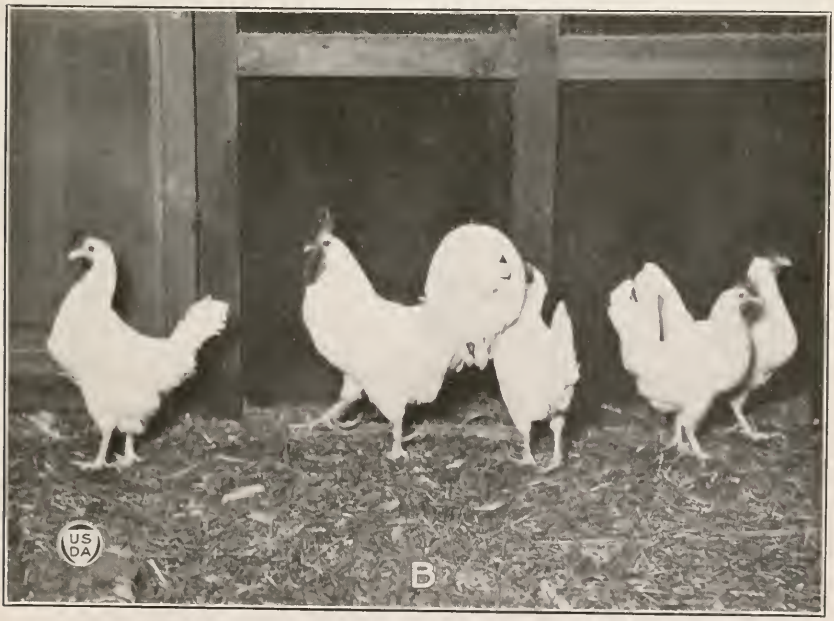


US
DA
A


US
DA
B

45-6[2]

PLATE 2

A.—Hen during second year of confinement.
B.—Seven and eleven months' old chickens grown in confinement.

ACIDITY OF CORN AND ITS RELATION TO VEGETATIVE VIGOR[1]

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INTRODUCTION

Some investigators are inclined to believe that there is but little variation in the cell-sap reaction of healthy plants of a given species, and that the hydrogen-ion concentration of each cell remains practically the same during its life (*2*).[2] The well-known facts that blood is so buffered that in a healthy individual its reaction never varies more than a few hundredths of a P_H unit from the norm, and that acidosis leads to profound physiological disturbances lend color to the supposition that the same might be true of plant juices. Consistent with this is the statement of Truog and Meacham (*16*), that for each species of plant there undoubtedly is a certain acidity which is most favorable for the life processes of that particular species. That this optimal reaction of the plant is susceptible to change is suggested by Clevenger (*5*) who says, "It seems possible that certain conditions may change the acidity of the plant juice sufficiently to produce an acidity which is unfavorable for the plant." Moreover, Haas (*8*) is led by his work on sweet clover, which showed a difference in vigor between the first and the second year's growth, and a higher hydrogen-ion concentration in the less vigorous plants, to ask the question, "Do the more vigorously growing plants of a species show a decrease in the actual reaction of their juice compared with that of less actively growing plants of the same species?"

This same question was very strongly suggested by the first few experiments of the present investigation, originally designed to determine whether or not there are varietal differences in the acidity of corn (maize) seedlings. A surprising variability in the acidity values [3] of plants of the same variety precluded conclusions on the latter question, but focused attention on an apparent correlation between the measurements and vegetative vigor.

PROCEDURE AND METHODS

Ten strains of Reid Yellow Dent corn (*Zea mays*), self-pollinated one year, were selected for these experiments by Dr. G. N. Hoffer at La Fayette, Ind. Dr. Hoffer, referring to the growth of these strains in Indiana, describes the plants of No. 31 and 972 as vigorous, without

[1] Accepted for publication June 25, 1923.
[2] Reference is made by number (italic) to "Literature cited," p. 468–469.
[3] Throughout this paper "acidity," unqualified, is used as a general term, including hydrogen-ion concentration and titratable acidity.

any apparent disease symptoms; No. 113, 129, and 326 as stunted, with spotted or streaked, or prematurely dead leaves; No. 150, 350, and 507 with various abnormalities of leaf and stalk, indicating susceptibility to disease; and No. 315 and 485 with a marked tendency to fall down. Four successive plantings of each of the 10 strains were made in greenhouses and a fifth in an outdoor plot, on the grounds of the Department of Agriculture at Washington, D. C. Growth conditions in these plots were not equally favorable for corn, hence the five series were strikingly different in vigor.

The plants, grown to the age indicated in the different experiments, were cut about 2 inches from the ground, and after being carefully freed from dust and dirt, were ground in a food chopper. The juice was squeezed from the pulp by hand through clean muslin and its acidity determined immediately. In the earlier experiments, the juice from the whole top was expressed as one sample, but later, when the quantity of material permitted, the juice from the leaves and stalks was expressed separately.

All hydrogen-ion determinations were made electrometrically. The major details of the method have been given in an earlier paper (*11*). The titrations of total acidity also were made electrometrically, a uniform procedure being followed in all cases. One-twentieth normal sodium-hydroxid solution was added, 1 cc. at a time, to a 10-cc. sample of undiluted juice by means of a burette, the tip of which was inserted through the cork in the electrode vessel. The P_H values corresponding to the potential differences resulting from each successive addition of alkali to the sample were plotted against the corresponding quantities of sodium hydroxid. The quantity required to bring the hydrogen-ion concentration to P_H 8.3, the turning point of phenolphthalein, was taken to represent the titratable acidity.

In order to discover whether a relation existed between the acidity of the juice and its density, specific-gravity determinations were made for each sample in the later experiments. For these determinations the juice was filtered and refiltered through a folded filter until clear, precautions being taken to prevent excessive evaporation. The density was determined by means of a small pycnometer in the form of a glass capsule with capillary intake and outlet tubes. The density is referred to water at the same temperature as that of the juice.

RESULTS

SERIES 1

The first set of plants was grown in medium to fine sandy loam [4] with a neutral reaction (P_H 7.0), in a greenhouse bench on which the soil was approximately 10 inches deep. By the sixth week, when the plants were cut, they had reached an average height of 50 cm. On the whole, the plants were normal and in good condition. Strains No. 113, 31, and 326, however, were less vigorous than the rest. Either two or three plants, depending on their size, were cut for each sample. One set of determinations was made in the morning, and in order to compensate for any error from differences in the time of cutting and order of expres-

[4] I am indebted to Mr. L. A. Hurst, of the Office of Soil Fertility Investigations, Bureau of Plant Industry, for the technical descriptions of the soils used in this investigation.

sion, duplicate determinations, but with their sequence reversed, were made on the same strains each afternoon. The results are given in Table I.

TABLE I.—*Acidity of the tops of corn plants of Series I, 36 to 39 days old,*[1] *in good condition, grown in the greenhouse in neutral sandy loam*

Strain No.	Date of determination (May, 1922).	Morning cuttings.		Afternoon cuttings.	
		Titratable acidity (cc. *N*/20 NaOH).	P_H	Titratable acidity (cc. *N*/20 NaOH).	P_H
972	27	4.5	5.68	4.4	5.68
129	25	4.9	5.54	5.0	5.61
507	27	4.8	5.60	5.5	5.58
350	24	5.0	5.55	5.6	5.56
485	24	5.0	5.59	6.0	5.46
150	27	5.6	5.52	5.7	5.55
315	26	5.8	5.48	5.7	5.44
113	25	6.1	5.42	5.6	5.45
31	25	5.8	5.56	6.6	5.50
326	26	[2](7.4)	[2](5.38)	6.6	5.44
Average		5.3	5.55	5.7	5.53

[1] Throughout this paper age is reckoned from the date of planting.
[2] Poorest plants in the plot, described as "stunted, leaf tips dead," not included in the averages.

The close agreement in the values obtained from morning and afternoon cuttings brings out the interesting fact that neither the hydrogen-ion concentration nor the titratable acidity changed measurably between 8 a. m. and 12.30 p. m. In those cases where the measurements were not the same, as many show a decrease as show an increase. Evidently the diurnal change in acidity, reported in many plants, is not marked in corn grown under these conditions.

SERIES II

The second set of plants was grown in another greenhouse in a sandy clay loam with a slightly acid reaction (P_H 6.5), and only 3 or 4 inches deep on the bench. This series, like the preceding one, reached an average height of 50 cm. by the sixth week, at which time the plants were cut. However, the stalks were spindling and the leaves narrow, whereas the plants of the first set had stout stalks and the broad leaves of normal plants of that age. As the plants were cut, they were weighed for comparison with those of subsequent series and found to average 44 gm. Green weight expresses more accurately than does height the rate of growth and health of the plant (7).

Either one or two plants, depending on their size, were taken for each sample and the stalks and leaves expressed together. Unfortunately, there was not sufficient material to make duplicate measurements on strains 326 and 113, and the plants of No. 485 and 350 were lost. The acidity determinations for this group of plants are recorded in Table II.

TABLE II.—*Acidity of the tops of corn plants of Series II, 37 to 44 days old, in poor condition, grown in the greenhouse in slightly acid sandy clay loam* [1]

Strain No.	Date of determination (June, 1922).	Titratable acidity (cc. N/20 NaOH).	P_H	Averages.	
				Acidity (cc. N/20 NaOH).	P_H
972	9 12	5.7 6.9	5.40 5.29	6.3	5.34
507	8 14 15	6.4 (8.5) 6.4	5.21 (5.10) 5.29	6.4	5.25
31	8 13 14	6.5 6.6 6.2	5.28 5.25 5.22	6.4	5.25
129	10 12 13	6.5 6.7 (9.0)	5.33 5.23 (5.10)	6.6	5.28
326	9	6.7	5.30	6.7	5.30
315	8 9 14 15	6.4 5.9 7.3 7.5	5.30 5.38 5.34 5.30	6.8	5.33
150	10 12 13 13	7.0 7.2 8.8 7.0	5.23 5.19 5.10 5.24	7.5	5.19
113	8	7.5	5.15	7.5	5.15
Average	....	6.8	5.26	....	....

[1] Measurements in parentheses are not included in the averages because they were made on exceptionally poor spindling plants, not typical of the row.

The outstanding feature of Table II is the high acidity of all the plants compared with the more vigorous ones of Table I. Additional evidence of an interrelationship of acidity and vigor of growth was afforded by the notes on relative development of some of the individual plants constituting the group. Some highly acid plants of 507 and 129, the measurements of which are inclosed in parentheses in the table and not averaged because the plants did not seem typical of the strains, were described, respectively, as "spindling stalk, narrow leaves, tips dying," and "short and spindling, poorest of all." Of the uniformly highly acid strains, No. 150 showed a tendency to become diseased in all the plantings, while No. 113 was nearly always at the bottom of the list in vigor.

SERIES III

To check the results given in Table II a third series had been planted between the rows of the second series before the plants were cut. The soil reaction was found to have become more acid, being P_H 6.2 as compared to P_H 6.5, the value obtained a month before. The plants grew even more poorly than those of Series II, having spindling stalks, and narrow leaves with dry, dead tips, the lowest dying early. The average height of the group did not reach 50 cm. until the seventh week when the plants were cut and found to have an average green weight of 44 gm., the same as that of the plants of Series II at six weeks. It was necessary, because of the small quantity of juice in each plant, to take from two to

five plants for each sample. There were not enough plants of No. 113 for even one determination. The results are given in Table III.

TABLE III.—*Acidity of the tops of corn plants of Series III, 44 to 56 days old, in very poor condition, grown in the same soil as Series II* [1]

Strain No.	Date of determination (July, 1922).	Titratable acidity (cc. N/20 Na OH).	P_H	Specific gravity.
507	8	6.9	5.23	1.0246
	8	(9.2)	(5.22)	(1.0253)
485	7	9.4	5.03	1.0221
	18	(12.3)	(5.01)	(1.0201)
350	7	9.5	5.16	1.0232
150	6	10.2	5.10	([2])
129	14	10.6	5.13	1.0248
315	11	10.8	5.06	1.0194
31	6	11.0	5.10	([2])
	11	(13.2)	(5.01)	(1.0272)
326	18	11.7	5.03	1.0246
972	14	12.3	4.97	1.0247
Average	....	10.3	5.09	1.0233

[1] Figures in parentheses are from the poorest, most spindling plants, not typical of the row, determined separately and not included in the averages, but given to show the greater acidity of the less vigorous plants.
[2] Not enough juice for determinations.

Comparison of the results reported in Table III with those in Table II shows that the plants have a markedly increased titratable acidity and hydrogen-ion concentration, although grown in the same soil. But these plants grew more slowly and were more spindling than their predecessors, besides showing an abnormal dying of the tips of the leaves. In Table III, as in the two preceding tables, are given in parentheses the values obtained from the poorer plants of each group which obviously were below the average in vigor. These data afford additional evidence of an interrelation of vigor and acid accumulation.

SERIES IV

The fourth series was a repetition of the first in the original greenhouse to see whether, on second trial, the growth conditions there would again produce the more vigorous, rapidly growing plants with their comparatively low acidities. Unused soil adjacent to that of the first plot was used for Series IV. A redetermination of the soil reaction showed that the soil on this bench remained approximately neutral, P_H 7.2.

The higher temperatures of June and July, as compared with those of April and May, probably were responsible for the fact that the plants of this series made an even more rapid growth than did those of Series I. At the end of only five weeks their average height was 80 cm. and their average green weight 126 gm. as compared with 50 cm. and 44 gm., the corresponding measurements for the unhealthy Series III at the age of seven weeks. The data are given in Table IV.

TABLE IV.—*Acidity of the tops of corn plants of Series IV, 32 to 35 days old, in good condition, grown in the greenhouse in sandy loam,* P_H *7.2*

Strain No.	Date of determination (July, 1922).	Titratable acidity (cc. N/20 NaOH).	P_H	Specific gravity.
485 (a)[1]	26	3.7	5.51	1.0135
485 (b)[1]	28	4.3	5.49	1.0153
113	27	4.3	5.49	1.0138
507	25	4.6	5.44	1.0148
326	26	4.7	5.56	1.0152
315	26	4.7	5.49	1.0132
350	27	4.8	5.55	1.0148
31 (a)[1]	28	4.8	5.43	1.0156
31 (b)[1]	28	5.6	5.34	1.0167
129	25	5.0	5.51	1.0145
972	27	5.1	5.51	1.0163
150	25	5.2	5.38	1.0152
Average	....	4.7	5.48	1.0149

[1] Strains 485 and 31 were uneven in growth, so the best plants of each row (a) were determined separately, and when compared with the rest (b) show a greater acid content in the less vigorous plants.

Table IV shows that the juice of the plants of this series had a hydrogen-ion concentration practically the same as that of the vigorous Series I and a slightly lower total acidity, while both the hydrogen-ion concentration and the total acidity were very much lower than in the weak spindling plants of Series II and III.

As these large, rapidly growing plants seemed so much more succulent than the spindling, slow-growing ones of Series III, it was not surprising to find the specific gravity measurements of their juice very much lower than those of that series. This fact suggests that the low acidity of the former compared to the latter may be due in part to a greater water-absorbing capacity of the rapidly growing plants, resulting in greater hydration of the tissues and dilution of the cell sap. It is interesting to note in this connection that Reed (*13*) found the concentration of the sap of certain trees to vary inversely with the rate of growth.

SERIES V

The fifth planting was in an outdoor plot for comparison with the greenhouse plants. The soil was a fine, sandy loam, slightly acid, with a P_H value of 6.6. The season was too far advanced for corn, the cool nights of September and October so inhibiting its growth that the plants were poor and more stunted than those of any other series. They were characterized by narrow leaves and by very short internodes. Many were leaning or fallen.

To facilitate comparisons of the acidity determinations of these slow-growing plants with those of vigorous ones of the same age, the averages of the acidity measurements for each strain are given in Table V alongside the corresponding values for plants of Series IV, which had been allowed to grow in the greenhouse until they were 8 weeks old.

TABLE V.—*Comparative acidities of the stalk and leaf juices of vigorous and of stunted corn plants, 8 weeks old*

[Series IV, vigorous, average height 150 cm., green weight 311 gm.
Series V, stunted, average height 60 cm., green weight 124 gm.]

Strain No.	Stalks.						Leaves.					
	Titratable acidity (cc. *N*/20 NaOH) of series—		P_H of series—		Specific gravity of series—		Titratable acidity (cc. *N*/20 NaOH) of series—		P_H of series—		Specific gravity of series—	
	IV	V	IV	V	IV	V	IV	V	IV	V	IV	V
507	2.5	4.8	5.68	5.22	1.0183	1.0172	9.8	9.3	5.45	5.46	1.0266	1.0225
485 [1]	3.0	8.0	5.41	5.03	1.0193	1.0197	10.2	11.6	5.37	5.40	1.0238	1.0296
31	3.3	5.6	5.50	5.25	1.0166	1.0190	9.4	10.3	5.52	5.46	1.0216	1.0258
972	3.5	4.3	5.35	5.25	1.0180	1.0148	10.4	10.9	5.43	5.47	1.0239	1.0253
350	2.3	6.9	5.69	5.19	1.0176	1.0202	9.0	11.9	5.43	5.42	1.0213	1.0261
326	2.5	8.2	5.59	5.16	1.0134	....	8.4	14.9	5.50	5.33	1.0204	1.0261
315	3.6	6.0	5.35	5.22	1.0166	1.0174	10.4	9.8	5.31	5.43	1.0234	1.0229
129	2.8	6.6	5.44	5.16	1.0143	1.0144	10.2	10.7	5.37	5.44	1.0236	1.0197
113	1.8	......	5.60	......	1.0194	....	9.6	......	5.40	......	1.0211	......
Average	2.8	6.3	5.51	5.19	1.0171	1.0175	9.7	11.2	5.42	5.43	1.0229	1.0248

[1] The plants of strain 485 in Series V appeared wilted when cut. This probably accounts for the unusually high acidity and hydrogen-ion concentration of the stalks and the high specific gravity of both stalk and leaf juice of those plants.

The data in Table V show that the stalk juices of the rapidly growing greenhouse plants of Series IV had a decidedly lower titratable acidity and hydrogen-ion concentration than did those of the stunted ones from the outdoor plot (Series V). That the stalks of these slow-growing plants are characterized by a relatively high acid accumulation rather than simply a high sap density due to an increased concentration of all the solutes is shown by the fact that the specific gravity figures bear no consistent relation to the acidity measurements.

In some of the strains there is no significant difference in the acidity values for the leaf juices of the good and the poor series. The acidity of the stalk appears to be affected in greater degree by the environment than does that of the leaves. There are some data reported in the literature pointing to this same fact. Thus, with different fertilizer treatments, Bauer and Haas (*3*) obtained variations in the actual acidity in the stalks of corn between P_H 5.31 and 5.95; and in the leaves between 5.31 and 5.49. Haas (*8*) found also that liming the soil decreased the actual and total acidities of the juice of the stems and petioles of red clover plants more than it did that of the leaves.

Consistent with the evidence that the concentration of titratable acid is higher in the leaves than in the stalk of the same plant are the specific-gravity measurements in Table V, which show that in every plant of each series, whether vigorous or stunted, there is a much higher sap density in the leaves than in the stalk. However, in all the stunted plants of Series V the hydrogen-ion concentration of the stalk juice was much higher than that of the leaves, although the titratable acidity was very much lower. In the vigorous plants of Series IV the lower concentration of hydrogen-ions accompanied the lower titratable acidity in the stalk, except in a few instances where the P_H values were practically the same. Furthermore, the ratio of the titratable acidity of the stalk to that of the leaves, which was 1 to 3 or 1 to 4 and sometimes even

smaller in the rapidly-growing plants of Series IV, was 1 to 2 or greater in the slow-growing plants of Series V.

To illustrate these differing characteristics of the plants of Series IV and V and also the extreme variations which are found in the acidity of plants of the same strain, some titration curves obtained with plants of No. 326 are given in figure 1. The data for stalk and leaves of a plant of less than average acidity from Series IV are plotted alongside those of a plant of more than average acidity from Series V.

The slope of the curve representing the stalks of the vigorous plants is steeper than that of the slow-growing ones and shows that the titratable acidity of the latter is more than three times that of the former. The curves representing the leaf acidities show a similar relation. The curve for the stalk juice of the poor plant starts below but crosses that of the

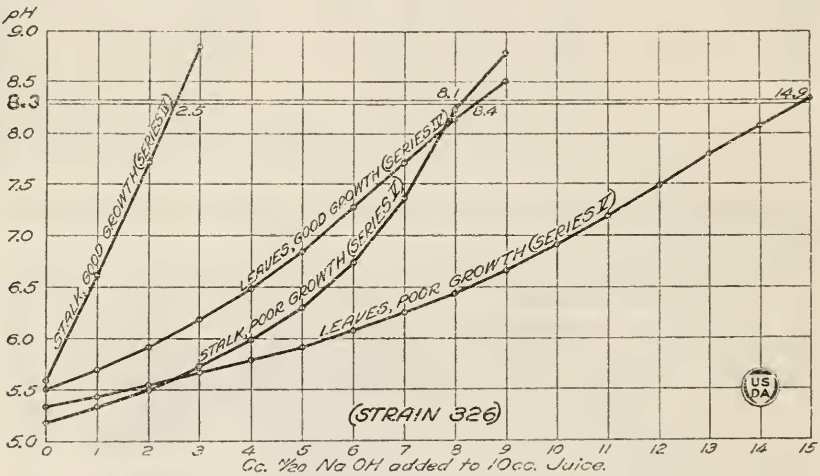

Fig. 1.—Electrometric titration curves of leaf and stalk juices of Reid Yellow Dent corn from vigorous plants (Series IV) from the greenhouse and stunted plants (Series V) from the field.

leaves, a relation shown by the curves for the leaves and stalk of every plant in Series V, but for none of the plants of the vigorous series.

The acidity data for strain No. 150 are not included in Table V because, in both Series IV and V, these plants appeared diseased and not comparable with the others. The leaves were crinkled, spotted, and dead at the tips, with the lower leaves prematurely dead. In Series IV, both the hydrogen-ion and titratable-acid concentrations in these leaves were definitely higher than in any others, being P_H 5.26 and 12.5 cc. (NaOH), respective, while the corresponding values for the stalks of the same plants were lower than the average concentration, being P_H 5.66 and 2.3 cc. Likewise, in Series V the hydrogen-ion concentration of the leaves of strain 150 seemed abnormally high with respect to the corresponding titratable-acidity measurement, but the acidity measurements of the stalks were much lower than those of any other strain in the plot. Moreover, in all the plants of every other strain in Series V the P_H value of the stalk was at least 0.2 below that of the leaves; yet in these diseased plants of strain 150 the figures were P_H 5.35 and 5.37, respectively.

DISCUSSION

Sufficient data have been presented to show that not only the titrable acidity, but also the hydrogen-ion concentration, of the expressed juice of corn plants is extremely variable. It has been shown that there exists a most striking dependence of acidity upon the reaction of the plant to its environment in so far as we can judge of this reaction by vegetative vigor. In Clevenger's (*6, p. 230*) article are discussed some of the ways in which he and others have found acid accumulation to be dependent upon internal and external factors.

It would not be amiss at this point to summarize the various P_H values reported in the literature for corn juice. Truog and Meacham (*16*) give 5.2 and 5.3 for the expressed juice of whole tops of corn plants from unlimed and limed soil, respectively. Haas (*8*) gives 5.19 and 5.48 for the tops of two different plantings, the first in Plainfield sand and the second in Colby silt loam. Bryan (*4*) found a variation from 5.1 to 5.2 in corn leaves, the plants being grown in sand and in solution cultures. Bauer and Haas (*3*), by applying various fertilizer treatments to quartz-sand cultures, obtained values varying from 5.31 to 5.49 in leaves and from 5.31 to 5.95 in stalks.

The range of P_H and titratable acidity values for entire corn tops obtained in the present investigation is given in Table VI, together with the more obvious environmental factors and estimates of the relative vigor of the plants. These values are the averages from Tables I, II, III, IV, and are brought together to facilitate intercomparisons. Each value is an average of all the measurements made on the respective series, each composed of 40 to 50 plants, and including an approximately equal number of plants of each of the 10 strains. Both the maximum and the minimum measurements of titratable acidity obtained in each series and given under "range" follow the same order as do the respective means, and make the reality and significance of differences between the latter more convincing.

TABLE VI.—*Summary of the effect of environment on the titratable acidity and hydrogen-ion concentration of tops of corn plants and the apparent correlation of these characters with vegetative vigor*

Series.	Months grown (1922).	Environment.				Relative vigor.	P_H.	Total acidity (cc. $N/20$ NaOH).		Specific gravity.
		Place.	Soil.	Soil reaction.	Outdoor temperature.[1]			Mean.	Range.	
				P_H	°C.					
I	April–May..	Greenhouse A deep bench	Sandy loam	7.0	62	Very good	5.5	5.5	4.4– 6.6	....
II	May–June...	Greenhouse B shallow bench	Sandy clay loam	6.5	69	Poor......	5.3	6.8	5.7– 8.8	....
III[2]	May–July...	.....do.........	.....do......	6.2	73	Very poor	5.1	10.3	6.9–12.3	1.0233
IV	June–July...	Greenhouse A deep bench	Sandy loam	7.2	76	Very good	5.5	4.7	3.7– 5.6	1.0149

[1] Averages of hourly temperature readings of the Weather Bureau for each day during the growth of the series.
[2] Grown in the same soil as Series II, planted between the rows before that series was cut.

Every strain of corn from these different plantings showed variations in the acidity measurements parallel to those of the group averages of Table VI. To illustrate graphically a typical instance, the electrometric titration curves obtained with plants of a representative strain, No. 315, from the four greenhouse plantings, are plotted side by side in figure 2.

Whatever may be the cause of the differences in the vegetative vigor of these series and the correspondingly wide variation in their cell-sap acidity, temperature may be excluded except for Series V, by reason of the unrelated sequence of good and poor series. The illumination was probably nearly the same for all the series, especially for those grown within the same greenhouse. Since corn has been found (*1, 3, 4, 9, 10, 14, 15, 17*) to grow well in soils and nutrient solutions of even higher acidity than that of the poorest plot ($P_H6.2$), soil acidity can probably be eliminated as a causal factor. From the facts that the vigorous Series I and IV were grown in deeper soil than were Series II and III, and, that the

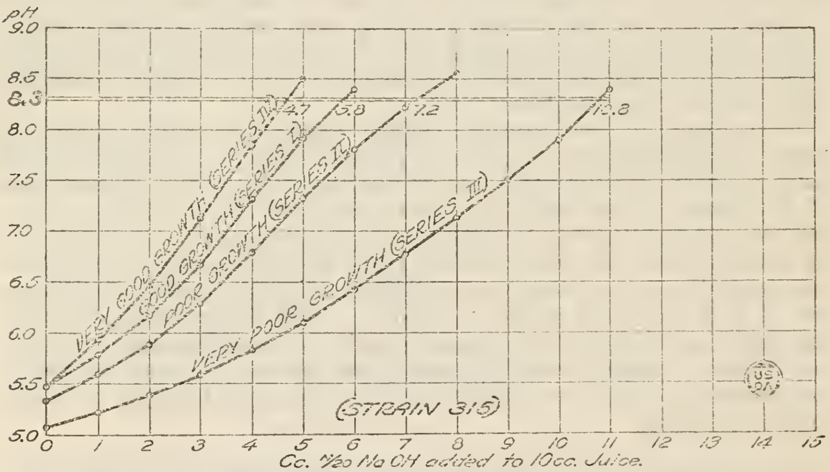

FIG. 2.—Electrometric titration curves of the juice of tops of Reid Yellow Dent corn from the four greenhouse plantings, illustrating the correlation of activity measurements with vegetative vigor.

poorest series, III, was planted in relatively shallow soil which had already been partly exhausted by one crop (Series II) it would appear that the inadequacy of some soil constituents was responsible for the spindling growth and high acidity of Series II and especially of III. Series V evidently was stunted as a result of low temperatures, since it is common experience that the cool nights of early fall prevent good growth of corn.

Consistent with the inverse relation between vegetative vigor and acid accumulation brought out by intercomparisons of the series or of the strains composing them, are those individual measurements (Tables I, II, III, IV) which show a lower acidity of the more vigorous individuals of the same strain as compared to the poorer ones growing beside them. This was true not only of the leaf juice but also of that of the stalk, the latter showing the correlation the more strikingly. Occasional departures from the rule occurred and from these exceptions it is evident that neither green weight nor height are invariably indications of relative acidity. A short stalky plant was occasionally less acid than a tall more slender one which had been judged the better plant.

In general, there is no parallelism between the magnitude of the individual readings of acidity and specific gravity in the tables. However, certain striking instances of high sap density accompanying high acid content strongly suggest some interdependence. Some examples of this relation are the wilted plants of strain 485 in Series V (Table IV). These external symptoms of a water deficit were accompanied by an unexpectedly high acidity and high specific gravity of the juices. The plants of strain 972 (Series IV) were so wilted, as the result of not having been watered the day before, that the leaves were limp and somewhat rolled. The titratable acidity measurements of both leaf juice and stalk juice were higher than the corresponding values for the unwilted plants of the same strain, and their specific gravity values were abnormally high, 1.0317 for leaf juice and 1.0246 for stalk juice. Further indication of a possible relationship of these characters is found in the fact that leaf juices always had a definitely higher sap density than the less acid stalk of the same plant. However, there are so many instances of juices which differ widely in acidity yet show no significant difference in specific gravity that difference in the hydration of the tissues obviously is not the only factor determining the magnitude of the difference in acidity values.

No conclusions can be drawn from these experiments as to the existence of varietal differences in acidity because the individual plants of each strain varied greatly in growth and vigor, and consequently in acidity, even when growing side by side in the same series. The varieties showing the most pronounced vigor, such as 507 and 485, usually were among the least acid in each series, suggesting that growth reactions to the environment rather than inherent acidity characters were responsible for the acid differences. Moreover, the varieties did not keep the same relative positions as to vigor in the different series, some reacting more poorly in one environment, while being among the best in another, thus recalling the work of Mooers (*12*) who found great differences in the relative yields of varieties on different soils. It would seem, therefore, that environmental factors, together with the capacity of the strain to respond to them as indicated by its vigor of growth, are more potent in determining cell-sap acidity then preexisting varietal acid tendencies.

SUMMARY

(1) The hydrogen-ion concentration of the tops of corn plants ranged from P_H 5.0 to 5.6 in the five plantings constituting these experiments, and was inversely correlated with the degree of vegetative vigor induced by the environmental conditions affecting the different plots.

(2) The titratable acidity of these tops varied correspondingly, the values ranging from an average of 10 cc. of *N/20* sodium hydroxid solution, required to neutralize 10 cc. of juice from plants of the most stunted plot, to 5 cc. (average) required to neutralize the same quantity of juice from the most rapidly growing plants.

(3) A lack of exact correlation between the magnitude of the acidity measurements and the specific gravity determinations of these juices shows that, in general, variations in sap density were not responsible for the variations in acid concentration. However, a number of striking instances of exceptionally high acidity values accompanied by correspondingly high density figures indicate that the water content of the tissues had, at times, a measurable effect on the acid concentration.

(4) The concentration of titratable acid was always higher in the juice of the leaves than in that of the stalk regardless of the plant's vigor. The hydrogen-ion concentration was higher in the leaves than in the stalk in the vigorous plants only; in the stunted plants it was greater in the stalks than in the leaves.

(5) In the slow-growing plants of the most stunted plot, the titratable acid concentration of the stalk was at least one-half that of the leaves; in the vigorous rapidly growing series this ratio was one-third or one-fourth and occasionally even lower.

(6) The specific gravity of the juice of the leaves was always higher than that of the juice of the stalk regardless of the plant's vigor.

(7) Environmental conditions produced far greater variations in the acidity of plants of the same strain than were ever found between plants of different varieties in the same environment and of equal vigor.

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BACTERIAL LEAFSPOT OF CLOVERS[1]

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INTRODUCTION

A leafspot disease of red clover, *Trifolium pratense*, quite unlike any of the several well-known diseases of this crop, was first noted in clover fields and on wayside plants in the vicinity of Madison, Wis., in 1916.[2] The next year the same disease was observed in the vicinity of Raleigh, N. C., not only on red clover but also on white clover, *Trifolium repens*, and on alsike, *Trifolium hybridum*, as well (Pl. 1, 2). This disease has been found in the District of Columbia and in neighboring Virginia and Maryland fields on the common red and white clovers. At Arlington, Va., it also occurs on *Trifolium repens*, var. *latum*, *Trifolium medium*, *Trifolium hybridum* and *Trifolium pannonicum* (Pl. 3). Preliminary microscopic examinations made of the Wisconsin material in 1916 showed that the disease was probably of bacterial origin. This conclusion was supported by the fact that isolation then made yielded a preponderance of similar white, bacterial colonies. Transfers from these proved uniformly to be pathogenic on red clover. A survey of the literature on clover diseases, both American and foreign, was accordingly made in an attempt to identify this bacterial leafspot disease. It was apparent from the few publications on this subject that this disease can not with certainty be identified as any of those previously described. Because of the economic importance of clover as a forage and hay crop, therefore, and of the lack of knowledge of this disease, independent investigations were undertaken at the University of Wisconsin, at the North Carolina Agricultural Experiment Station, and in the Laboratory of Plant Pathology at Washington, D. C. These have for the most part been prosecuted independently, with numerous interruptions at each place,

[1] Accepted for publication July 3, 1923.

[2] The investigations of this clover disease have been involved with those of related diseases of other legumes at Wisconsin and elsewhere, and acknowledgement is due to several associates aside from the authors. The disease on red clover was first observed by Dr. A. G. Johnson and Dr. C. S. Reddy in 1916, and the first isolation of the causal organism was made by Doctor Reddy. These two men and Dr. F. R. Jones have supplied further data on the distribution on this host. The details of the Wisconsin studies during 1917–18 passed into the hands of Miss Florence Coerper and were carried on in conjunction with her related investigations of soybean bacteriosis. In 1919 Miss Maude Miller (Mrs. Williamson) succeeded her in handling these details. When it was learned that Dr. F. A. Wolf in North Carolina and Miss Lucia McCulloch in Washington, D. C., had each independently found and studied the same disease with supplementary host range, it was decided to correlate the results for joint publication. The final development of these plans has necessitated conferences, somewhat delayed publication, and in places has encumbered the text with details which might otherwise have been omitted. It is, however, believed that the scientific worth and convenience of such correlation as compared with independent reports justifies joint publication. The only personal regret to me in the outcome is that the position of my own name as senior author fails properly to indicate the indebtedness to my younger associates for most of the details of workmanship.—L. R. J.

and the present paper, which embodies a correlated report of these results, has finally been compiled at the suggestion of the senior author. The aim has been to give an adequate account of the disease as observed independently at the three locations, together with the evidence as to its etiology and the characters of the pathogen.

HISTORY AND DISTRIBUTION

Observations throughout the period covered by these investigations show that the disease appears every year throughout the growing season but is not usually the cause of serious damage. It is not conspicuous in the field except in periods when moisture conditions are especially favorable for its development. Observations made in Wisconsin, both in the vicinity of Madison and elsewhere in the State, in various parts of Iowa and Indiana, and at a number of widely distant points in North Carolina, also in Virginia, Maryland, and the District of Columbia, indicate that this disease is of very general occurrence. It doubtless occurs widely at least in the United States and has heretofore escaped notice because of its confusion with other clover leafspot diseases.

Mention has been made in previously published accounts of several bacterial diseases of clover. The first of these was described in 1896 by Voglino (*7*).[3] This account deals with a leafspot disease which was rampant in several provinces of Italy on white clover, was common on *Trifolium resupinatum*, but rarely attacked red clover. It caused the formation of definite, usually numerous small black spots, most evident on the lower leaf surface. The floral parts were also involved and presented a similar diseased appearance. Voglino concluded that this disease not only materially reduced the yield of forage but also rendered it distasteful to grazing animals. The disease was ascribed by him to a bacterial parasite which he described as a new species, *Bacillus trifolii*. So far as can be determined, this disease has thus far not been reported outside of northern Italy.

Although this Italian disease and the bacteriosis under discussion have certain characters in common, they differ in several important features. In the first place, the cultural characters, then not regarded as of special significance but now considered essential to the exact description of species of bacteria, are almost wholly lacking in Voglino's description. Further, *Bacillus trifolii* is described as 0.5 to 5.0μ in length by 0.2 to 0.5μ in width and is thus more slender than the organism associated with the disease under discussion. In addition, it forms, as shown in Voglino's figures 10 and 11, drumstick-like spores, a character not possessed by the organism herein described. Then, too, even in the absence of type specimens with which to make direct comparison, his figure 1, which illustrated the character of the lesions on the foliage, leaves no doubt that Voglino's disease is distinct from the one dealt with in the present paper.

A disease reported from Italy in 1913 by Baccarini and his associates (*1*) under the name "incappucciamento" manifests itself in a very different manner from the leafspot disease described by Voglino. Since this malady was so severe in northern Italy as to cause a failure of the crop, a commission was assigned to investigate it. Doctor Bargagli, bacteriologist for this commission, believed it to be of bacterial origin. This clover disease is characterized by a general stunting of the above-ground portions of the plant, as indicated by the dwarfed, yellowish

[3] Reference is made by number (italic) to literature cited, p. 490.

leaves and inhibition of development of new vegetative and floral organs. Baccarini's brief account neither names the causal organism nor includes a description of it, but the disease manifestly has no relation to the American bacterial leafspot.

In his comprehensive account of the diseases of the clovers in Russia, Jaczewski (3) makes no mention of any form of clover bacteriosis in that country, but indicates his familiarity with those in Italy by reference to the two listed above. Aside from these investigations, bacteriosis of clover in Europe appears not to be recorded, and the only account of its occurrence in America is that included in Manns' (5) studies of the "streak" disease which is especially prevalent upon the sweet pea. This disease, which is attributed to *Bacillus lathyri* Manns and Taubenhaus, is recorded also as occurring on clover and certain other legumes. This bacillus is a yellow organism with very different morphological and cultural characters from the clover organism under present consideration. Furthermore, Manns' disease is characterized by longitudinal stem lesions, which may later involve the petioles and leaves, thus presenting symptoms very different from those of the bacterial leafspot described by the writers.

It is entirely probable that bacterial leafspot has been confused with the several fungous leafspots which, especially in their later stages, may be difficult to differentiate without the aid of a microscope. At any rate, it has not hitherto been clearly recognized and there are no unquestionable previous records of its occurrence.

APPEARANCE OF THE DISEASE

SEASONAL DEVELOPMENT

A week or two after the first clover leaves become green in spring bacterial leafspot begins to appear. In Wisconsin the infections have usually been noted in late May and early June upon the young leaflets. Even though clovers remain green throughout the winter in North Carolina, no evidence of bacterial leafspot has been found until the warm days of May. In the vicinity of Washington, D. C., infections have not been observed until late in May. If the weather continues moist, the disease progresses upward with the growth of the plant so that at blossoming time even the uppermost leaves may be conspicuously spotted (Pl. 1).

There is no evidence that the disease is systemic, and new infections may appear at any time when temperature and moisture conditions are favorable. In some seasons severe infection appears on the young growth following the cutting of the first crop and may be found commonly in the fields as late as September and October. In 1916 the disease was very abundant on both the first and second crops of clover at Madison, while in 1919 hot, dry weather checked the disease in early June, and scarcely any new infections appeared after that time. At Arlington, Va., the disease has appeared in three successive years on the second crops of clover.

SYMPTOMS

As the name implies, the most conspicuous lesions of this disease appear on the foliage, although stems, leaf petioles, stipules, and flower pedicels are also seriously involved. The presence of tiny translucent

dots on the lower leaf surface is the first indication of infection. These lesions enlarge and become more or less angular, since they are quite sharply delimited by the veins. Meanwhile, the centers of the spots become inky black; but the margins retain the water-soaked character even at maturity. The centers of old lesions on desiccation become dark brown and parchmentlike. The tissues outside the translucent border of the lesion become chlorotic, and badly spotted leaves are distinctly yellowish. The infections may be so abundant that large, irregular, dead areas are formed. There is a tendency for the central tissues of old

FIG. 1.—Leaf of red clover, *Trifolium pratense*, with bacterial leafspot, natural infection. This shows the characteristic form, size, and distribution of lesions, both marginal and interior, the latter generally intervenous. (Drawing from nature by Charles Drechsler.)

lesions to crack or to fall away. In consequence, if these lesions are marginal, the leaves present a frayed and torn appearance. Abundant spotting causes the entire leaflet to become dry or to shed prematurely.

Under very humid conditions a bacterial exudate may appear on the lower leaf surface. This exudate has the appearance of a thin film or of small, milky, glistening droplets. If diseased leaves are kept for 12 to 24 hours in a moist chamber, the bacterial slime will have exuded to form larger droplets from which the pathogen may be obtained in pure culture. On drying, this exudate becomes a thin, incrusting film.

Infection of petioles, stems, stipules, and flower pedicels are of less common occurrence than infection of the leaflets, and the lesions are less characteristic. Those of the petiole and stem appear as dark, elongated, slightly sunken spots. On stipules of *Trifolium pannonicum*, long, inky-black lines were produced (Pl. 3, D). The translucent margin is less pronounced than on leaf lesions.

CAUSAL ORGANISM

ISOLATION

Bacteria are abundant in the lesions, and at each of the three laboratories, Madison, Raleigh, and Washington, the identical organism has been isolated repeatedly. In some cases (Wisconsin, Washington) isolation has been preceded by surface rinsing of the tissues with alcohol or mercuric chlorid solution; in others (North Carolina) direct maceration was made of young lesions in sterile water. One of the early Wisconsin isolations from red clover, termed 1916–II, was first proved to be pathogenic, then used in the earlier detailed cultural studies, and more recently has been compared with the subsequent isolations from Madison red clovers, termed 1919–I and 1920–III. These three have been carried critically through comparative cultural studies at Madison and have throughout the experiments shown essentially identical characters.

At Raleigh several like strains were isolated from red, white, and alsike clovers and their pathogenicity proved by successful inoculation and reisolation. One strain from each of these host species was used for cultural studies at Roleigh in 1922. Other strains from isalations of previous years were also used in comparative studies with the Wisconsin strain 1920–III.

FIG. 2.—Bacterium causing leafspot of red clover. Casares-Gil flagella stain.

At Washington isolations were made from natural infections on the red, the common white, the large-leaved white (var. *latum*) and alsike clovers, also from *Trifolium medium* and *T. pannonicum*. All the strains were compared and found to be practically identical in morphological and cultural characters and in ability to reproduce the disease not only on the original host but also on other clover species. Three strains were selected for the further studies: Strain 3 from white clover, strain 4 from red clover, and strain M, a reisolation from red clover inoculated with strain 3.

MORPHOLOGY

The pathogen is a small rod with rounded ends, usually occurring singly but tending in bouillon to form short chains. It stains readily with Ziehl's carbol fuchsin, anilin gentian violet, and Loeffler's methylene blue. When stained from 24-hour potato-agar cultures with methylene blue, the cells are 1.7 by 0.6μ with extremes in length from 1.2 to 3.0μ and in width from 0.4 to 1.0μ.

The organism is motile by means of from one to four unipolar flagella which are about 2 to 3 times the length of the cell (fig. 2). Flagella have been demonstrated in agar cultures 1 to 4 days old by the methods of Casares-Gil, of Duckwall, and of Loeffler, as modified by Shunk.

Endospores and marked involution forms have not been noted. With the use of Ribbert's dahlia capsule stain, both the Wisconsin and the eastern strains tested have shown a thin but definite enveloping sheath. The organism is decolorized by Gram's method.

CULTURAL CHARACTERS

The cultural characters as here described were first worked out at Madison with the Wisconsin red-clover organism. It has therefore seemed expedient to designate that as the type strain, and unless otherwise specified the following descriptive characters are based on these Wisconsin studies. In all cases, however, the results obtained in the Raleigh and Washington studies are in essential agreement with these. In the Wisconsin studies the nutrient broths contained 1 per cent peptone and 0.3 per cent Liebig's beef extract; the nutrient agars the same with addition of 1.8 per cent of bacto-agar; the peptone broths either 1 or 2 per cent of Difco peptone. The cultures were kept in dark, well-ventilated incubators held at approximately 25° C. Color determinations follow the Ridgway color standards.[4]

Agar poured plates.—On nutrient agar, colonies appear after 48 hours, and in 5 days have attained a diameter of 2 to 3 mm. They are circular in outline with entire margins, convex or slightly umbonate, smooth, glistening, and opaque white. Submerged colonies remain small and are lenticular in shape. The agar is unchanged in color and no odor is developed.

On potato agar[5] growth is more abundant, and in 5 days the colonies are 3 to 4 mm. in diameter. They are circular, having entire margins, a surface which is rugose at the center, and a tendency toward contoured markings at the periphery (Pl. 5). The colonies are white and butyrous in consistency.

Agar stabs.—On nutrient agar the surface growth was at first moderate with a faintly beaded outline along the line of the stab. Later it became more abundant at the surface with colony characters like those on plates. A decided fluorescence was apparent in cultures 2 weeks old.

The growth on potato agar was limited to the upper one-half inch of the line of stab as a faintly beaded line. The surface growth was moderate, convex, smooth, glistening, and opaque grayish white. The colonies become larger than in poured plates but are never larger than 3 to 4 mm.

Agar slants.—From 5 to 10 days are required on nutrient agar to secure an abundant growth. It is then filiform, spreading, with an entire margin toward the base of the stroke, glistening, and translucent. When one removes a portion of the growth with a platinum needle, it is found to adhere in a butyrous opaque mass. A slight fluorescence appears in the medium in 2-week-old cultures.

On potato agar more abundant growth occurs which is dull and raised. The surface is rugose with radial folds extending outward from the line

[4] Ridgway, Robert. color standards and color nomenclature. 43 p. 53 col. pl. Washington, D. C. 1912.

[5] Potato agar as used in the Wisconsin laboratory contained 1,000 cc. water, 200 gm. potato, 20 gm. dextrose, and 18 gm. bacto-agar.

of the stroke to the contoured margin (Pl. 6). No odor is developed, and the agar is unchanged.

GELATIN PLATES.—On gelatin plates visible growth was slow, but after five days the colonies were 3 to 4 mm. in diameter with a smooth surface and entire margin. They were finely granular within, especially toward the center. The organism is not capable of liquefying the substratum.

GELATIN STABS.—Best growth occurs at the surface in stab cultures, with neither liquefaction nor discoloration of the medium.

POTATO CYLINDERS.—On steamed potato cylinders growth is first manifest by a faintly yellowish white, spreading streak. It becomes abundant within six to eight days, but remains flat, grayish, and gelatinous in consistency. The cylinder along the line of growth becomes smoky gray. No marked dissolution of the potato tissue occurs, and the potassium iodid test indicates a weak diastasic activity only in 3-week-old cultures.

MILK.—Plain sterilized milk turns creamy in color after two weeks; after five weeks a soft curd forms which slowly separates into whey and a rather firm curd which was not digested at the end of four months. The whey becomes alkaline with litmus as an indicator.

LITMUS MILK.—Lavender-colored litmus milk changes rather rapidly through dark plumbago blue at the end of 5 days, deep Dutch blue at the end of 10 days, to light Tyrian blue at the end of 2 weeks. Curd begins to form soon afterwards and becomes dull tan, whereas the whey is dark blue.

METHYLENE BLUE IN MILK.—Decolorization had been completed in two weeks, but slowly returned with the separation of curd and whey. The color reappeared in the whey.

BLOOD SERUM.—Stroke cultures showed in two to three days' moderate growth, spreading, flat, smooth, and glistening with an echinulate margin. No liquefaction occurred and no discoloration of the medium was noted.

SYNTHETIC MEDIA.—Cohn's solution does not appear to support growth.

In Fermi's solution it soon develops turbidity, and after three days sufficient growth has taken place to produce a milky white cloudiness. After five days a thin viscid pellicle will have formed. The medium gradually takes on a bluish green fluorescence.

In Uschinsky's solution two or three days' growth results in a milky-white cloudiness. Delicate, flocculent pellicle-like growths appear at the surface. The fluorescence which gradually develops is not so marked as in Fermi's solution.

DIGESTION OF CASEIN.—Poured plate cultures in casein agar after a week's incubation had developed colonies 5 to 6 mm. in diameter. When these cultures were tested by flooding with a 1 per cent solution of hydrochloric acid to precipitate the casein, it was found that a narrow zone surrounding each colony remained transparent, whereas the remainder of the plate was milky white. This indicates that the organism is able to digest the casein in the zone immediately surrounding the colonies.

AMMONIA PRODUCTION.—Cultures in peptone broth and beef extract-peptone bouillon 7 days old showed ammonia to be present when tested with Nessler's reagent.

REDUCTION OF NITRATES.—Tests were made with Trommsdorf's reagent in tube cultures containing 2 per cent peptone broth to which 2

per cent potassium nitrate had been added. Satisfactory growth occurred, but no indication of nitrites was secured when the test was applied at the end of 7, 14, and 21 days.

TOLERATION OF SODIUM CHLORID.—Tubes of neutral beef extract-peptone bouillon containing 0.5, 1, 1.5, 2, 3, 4, and 5 per cent of pure sodium chlorid were used in this test. Best growth occurred in the presence of 0.5 per cent sodium chlorid. Higher concentrations of salt were progressively inhibitive, since the growth in 1 and 1.5 per cent was less than in 0.5 per cent, and that in 2 per cent very slight and with no visible clouding in higher concentrations.[6]

GAS PRODUCTION.—These tests were conducted by using fermentation tubes filled with solutions prepared as follows: A 2 per cent peptone solution was used as the base for six solutions made by adding 2 per cent of the following carbon compounds—glycerin, mannite, lactose, maltose, dextrose, and saccharose. These were prepared in sets of 14 each, and were then sterilized and incubated to determine their sterility before inoculation. Eight were then inoculated with Wisconsin type strain 1916–II, three with strain 1919–I, and three with 1920–III. They were not disturbed during the period covered by the test. No gas was produced in any case, and growth was sharply limited to the open arm in all media with each of the strains.

The media for another series were prepared by using a bouillon consisting of 1 per cent Difco peptone, 0.3 per cent Liebig's beef extract, and 0.5 per cent sodium chlorid, as a stock solution. The same carbon compounds were employed and they were prepared separately in 20 per cent solutions in distilled water. These solutions were sterilized at 10 pounds pressure for 10 minutes. The sugars were then added to the bouillon under aseptic conditions and the solutions poured into sterile fermentation tubes. After incubation for 48 hours to determine their freedom from contamination, they were inoculated with strain 1920–III and a single strain from each of three species of clover. Five tubes were used in each set of sugars with each strain. No gas was developed, and no growth took place in the closed arm, results entirely in accord with the previous test.

CARBON METABOLISM [7]

AGAR WITH SUGARS.—Agar was employed in the qualitative studies to determine the production of acid from the several common carbon compounds. It was prepared by adding to flasked and sterilized bacto-agar, cooled to 60° C. and adjusted colorimetrically to P_H 7.4, sufficient of the stock solutions of the carbon compounds to make 1 per cent of the sugar to be tested. Phenol red was added as an indicator. Before the agar had solidified it was poured into sterile test tubes for use in stab cultures. Dextrose, saccharose, lactose, maltose, and glycerin were tested in this manner. After the tubes of media had been incubated sufficiently long to determine freedom from contamination, they were inoculated in sets of five cultures on each sugar with Wisconsin strain 1920–III, and with a strain from red clover, one from white, and one from alsike from Raleigh, N. C. The red color disappeared with all strains in dextrose agar and in saccharose agar within five to seven days, indicating acid production,

[6] At Washington in beef infusion bouillon+13 Fuller's scale, P_H 6.7, with sodium chlorid added, the Wisconsin as well as the Virginia organisms gave growth in concentrations up to 4 per cent sodium chlorid.

[7] These statements concerning carbon metabolism are based on studies made by F. A. Wolf at Raleigh, N. C. The results with all carbohydrates tested in Wisconsin were in agreement with these.

but all cultures became progressively more alkaline in the other compounds.

BOUILLON WITH SUGARS.—Liquid media were used to follow the progressive changes in hydrion concentration produced during the fermentation of the several carbon compounds. Plain bouillon consisting of 1 per cent Difco peptone, 0.3 per cent Liebig's beef extract, and 0.5 per cent sodium chlorid served as a stock solution. The sugars prepared separately were added to this bouillon to make a concentration of 1 per cent.

During the course of this investigation the organism causing bacterial leafspot of clover has been used in parallel cultural studies with *Bacterium glycineum* and *Bact. sojae* from soybean and found to possess many characters in common with them. These studies as regards the soybean organisms have been reported in other papers (*2*, *6*, *10*). In view of the fact that it was found to be impossible to separate the clover organism from *Bact. sojae* on the basis of its fermentative ability on media containing the common sugars, it was believed that the "rare" sugars could be employed with success in distinguishing them. Accordingly, in addition to preparing bouillon containing dextrose, saccharose, lactose, maltose, or glycerin in concentrations of 1 per cent, a series of tubes were prepared with bouillon containing 1 per cent of either of the following: The pentoses, xylose and arabinose; the methylpentose, rhamnose; the hexoses, levulose and galactose; the alcohols, mannitol and dulcitol; the polysaccharids, inulin and dextrin; and the glucoside, salicin. Three strains of the pathogen, one from red, another from white, and the third from alsike clover, were used in one of these series. The tubes containing the cultures to be tested were compared colorimetrically with standard buffer solutions. Changes in reaction resultant on fermentation were followed by readings at 24-hour intervals. A considerable number of cultures of each strain with each sugar was employed so that several different tubes could be used in each consecutive reading. The results of these fermentation tests are assembled in Tables I, II, and III.

TABLE I.—*Fermentation of various carbon compounds in plain bouillon by the organism from alsike clover* (initial P_H 7.2)

Carbon compound.	Age of culture and P_H concentration.					
	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.
	P_H	P_H	P_H	P_H	P_H	P_H
Dextrose	7.2	7.0	6.6	6.2	6.4	6.6
Saccharose	7.2	7.2	7.0	6.6	6.2	6.6
Lactose	7.2	7.2	7.2	7.4	7.4	7.6
Maltose	7.2	7.2	7.2	7.2	7.2	7.4
Glycerin	7.2	7.2	7.2	7.4	7.4	7.6
Inulin	7.2	7.4	7.6	7.6	8.0	8.0
Dextrin	7.2	7.2	7.2	7.4	7.4	7.6
Arabinose	7.2	7.2	7.2	7.4	7.4	7.6
Xylose	7.2	7.2	7.2	7.4	7.4	7.6
Levulose	7.2	7.2	7.2	7.4	7.4	7.6
Dulcitol	7.2	7.4	7.4	7.6	7.8	7.8
Mannitol	7.2	7.4	7.4	7.4	7.6	7.6
Salicin	7.2	7.2	7.2	7.2	7.4	7.6
Galactose	7.2	7.2	7.2	7.2	7.2	7.4
Rhamnose	7.2	7.2	7.2	7.4	7.4	7.6

TABLE II.—*Fermentation of various carbon compounds in plain bouillon by the organism from red clover* (initial P_H 7.2)

Carbon compound.	Age of culture and P_H concentration.					
	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.
	P_H	P_H	P_H	P_H	P_H	P_H
Dextrose	7.2	7.2	7.0	6.6	6.2	6.6
Saccharose	7.2	7.0	6.8	6.6	6.6	7.0
Lactose	7.2	7.4	7.4	7.4	7.4	7.6
Maltose	7.2	7.2	7.4	7.4	7.4	7.6
Glycerin	7.2	7.2	7.4	7.6	7.6	7.8
Inulin	7.2	7.4	7.6	7.8	7.8	7.8
Dextrin	7.2	7.4	7.4	7.4	7.4	7.6
Arabinose	7.2	7.2	7.4	7.4	7.4	7.6
Xylose	7.2	7.2	7.4	7.4	7.4	7.6
Levulose	7.2	7.2	7.2	7.2	7.4	7.6
Dulcitol	7.2	7.4	7.4	7.4	7.4	7.6
Mannitol	7.2	7.4	7.4	7.4	7.4	7.6
Salicin	7.2	7.4	7.6	7.6	7.6	7.8
Galactose	7.2	7.2	7.2	7.2	7.4	7.4
Rhamnose	7.2	7.2	7.4	7.4	7.4	7.4

TABLE III.—*Fermentation of various carbon compounds in plain bouillon by the organism from white clover* (initial P_H 7.2)

Carbon compound.	Age of culture and P_H concentration.					
	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.
	P_H	P_H	P_H	P_H	P_H	P_H
Dextrose	7.2	6.6	6.4	6.2	7.2	7.4
Saccharose	7.2	7.0	6.8	6.4	6.4	7.0
Lactose	7.2	7.4	7.6	7.6	7.8	8.0
Maltose	7.2	7.4	7.6	7.6	7.8	7.8
Glycerin	7.2	7.4	7.6	7.8	7.8	8.0
Inulin	7.2	7.4	7.4	7.6	7.8	7.8
Dextrin	7.2	7.2	7.4	7.4	7.6	7.8
Arabinose	7.2	7.2	7.4	7.4	7.6	7.8
Xylose	7.2	7.4	7.4	7.6	7.6	7.8
Levulose	7.2	7.4	7.6	7.6	7.8	8.0
Dulcitol	7.2	7.4	7.4	7.6	7.6	8.0
Mannitol	7.2	7.4	7.6	7.8	8.0	8.0
Salicin	7.2	7.4	7.6	7.8	7.8	7.8
Galactose	7.2	7.2	7.4	7.4	7.6	7.8
Rhamnose	7.2	7.4	7.4	7.4	7.6	7.8

These results so far as the utilization of dextrose and saccharose is concerned confirm those with agar. Furthermore, it is evident that these strains of bacteria represent a single species since they are identical in ability to bring about the hydrolysis of dextrose and saccharose. There is a reversal of reaction with these two carbohydrates due, as has been shown by Wolf and Foster (*11*) with certain other forms, to a lack of sufficient fermentable sugar to permit the attainment of the final maximum hydrion concentration. The clover organism is unable to utilize the other sugars.

After having established the fermentation relations of the clover organism, another series of tests was initiated to determine points of similarity and difference between it and *Bacterium sojae*. In these tests clover strain 1920–III and the soybean organism were grown in comparative cultures with the results shown in table IV.[8]

TABLE IV.—*Comparative carbohydrate fermentations of clover strain 1920–III and Bacterium sojae in bouillon containing 1 per cent sugar (initial* P_H *7.4)*

Carbon compound.	Age of cultures, organism, and P_H concentration.					
	3 days—Organism.		5 days—Organism.		7 days—Organism.	
	Clover.	Soybean.	Clover.	Soybean.	Clover.	Soybean.
	P_H	P_H	P_H	P_H	P_H	P_H
Dextrose	7.0	6.8	6.4	6.6	6.8	7.2
Saccharose	7.0	6.8	6.2	6.4	6.6	6.6
Lactose	7.4	7.6	7.6	7.8	7.8	8.0
Maltose	7.4	7.6	7.4	7.6	7.6	7.8
Glycerin	7.4	7.4	7.6	7.6	7.8	7.6
Inulin	7.4	7.4	7.6	7.8	7.8	8.0
Galactose	7.4	7.4	7.6	7.6	7.6	7.6
Arabinose	7.4	7.2	7.6	6.6	7.8	5.8
Xylose	7.4	6.8	7.6	6.0	7.6	5.4
Levulose	7.4	7.2	7.6	7.0	7.6	6.8
Dulcitol	7.6	7.4	7.8	7.6	8.0	7.8
Salicin	7.4	7.4	7.6	7.6	7.8	8.0
Rhamnose	7.4	7.4	7.6	7.6	7.8	8.0

Strain 1920–III is able to ferment dextrose and saccharose alone and is therefore identical in carbon metabolism with the other strains from clover. *Bacterium sojae* is able in addition to hydrolyze arabinose, xylose, and levulose, and on this basis, together with inoculation studies to be reported subsequently, is regarded as specifically distinct from the bacterial leafspot of clover.

It might be pointed out at this point that very little use has hitherto been made of the rare sugars in cultural studies of plant pathogens. This is due in part, no doubt, to the fact that host species with more than a single known bacterial disease are less common than with animals. Rare sugars are recognized as invaluable, however, by investigators of forms pathogenic to animals in the separation of closely related species. Winslow, Kligler, and Rothberg (*8*), for example, have pointed out the significance of xylose and rhamnose fermentations in the colon-typhoid group. They found that xylose can be employed in separating the typhoid and paratyphoid B groups from the dysentery and paratyphoid A groups, while rhamnose furnishes a good basis of distinction between the paratyphoid (A and B) groups, and the typhoid and dysentery bacilli. Koser (*4*) has noted that *Bacillus suipestifer* is unable to utilize the disaccharid trehalose, whereas *B. paratyphosus*, *B. schottmülleri*, and *B. enteriditis* can ferment it with the formation of both acid and gas.

[8] At Washington comparative tests were made with the Virginia and the Wisconsin clover organisms in sugar media. Both fermented dextrose and saccharose, but the Virginia cultures produced less acid than the Wisconsin cultures. Neither of these clover strains fermented lactose, maltose, mannitol, galactose, or glycerin.

Certain other investigations as instanced by those with soybean blights (*6*) and tobacco wildfire and angular leafspot (*11*) have shown the value of rare sugars in differentiating closely related plant pathogenic bacteria. No doubt recognition of this value will be further appreciated as a larger number of bacterial diseases of plants come to be known.

TOLERATION OF ACID AND ALKALI[9]

Peptonized beef broth was adjusted with normal solutions of sodium hydroxid and hydrochloric acid as shown in Table V. Titration was made of each grade of bouillon with phenolphthalein for Fuller's scale value, and the P_H value of each was determined by both the colorimetric and the electrometric methods.

Two stock bouillons were used, one a 0.3 per cent solution of Liebig's beef extract, the other a fresh beef infusion. To each of these was added 1 per cent Difco peptone. All the beef-extract media were from the same stock (+8 Fuller's scale value, P_H value 6.7) and all the beef infusion was from one stock (+22 Fuller's scale value, P_H value 6.4). The initial tests of the media and the inoculations were made on the same day. Inoculations were made from 24-hour-old beef-bouillon cultures, all in vigorous condition, having been grown under favorable conditions for a number of days before transfers were made to the bouillon used for inoculations. The cultures were from the following sources:

VIRGINIA CLOVER.—Strain 3, from white clover, natural infection, September, 1921; strain 4, from red clover, natural infection, August, 1921; strain M, from red clover inoculated with strain 3. Reisolated December, 1921.

WISCONSIN CLOVER.—Cultures from the Wisconsin laboratory, No. 276, isolated 1920, and No. 210, isolated 1916.

BACTERIUM SOJAE.—Strain L, a culture received in November, 1921, from the North Carolina laboratory; strain 8, a reisolation made in December, 1921, at Washington from soybeans inoculated with strain L.

BACTERIUM GLYCINEUM.—No. 270, isolated from soybean in 1919, culture received November, 1921, from the Wisconsin laboratory.

These several cultures of the various organisms had previously been compared with others from the same host plant and had been found entirely representative, and all, except the Wisconsin clover cultures, were actively pathogenic. The Wisconsin cultures were perhaps from earlier isolations and produced in the Washington experiments in 1921 and 1922 only weak infections.

Bouillons for this test were inoculated in February, 1922. The inoculated tubes were kept in a dark, well-ventilated room at 26 to 27 C.

The results of this test as shown in Table V indicate that the Virginia clover strains are less tolerant of alkali than any of the other organisms. The Wisconsin clover strains show only slightly less tolerance of alkali than the soybean organisms. A difference in the age of the Wisconsin and Virginia clover strains may account for their different reaction in alkaline media.

[9] The following statements and table on toleration of acid and alkali are based on studies made by Lucia McCulloch at Washington, D. C.

TABLE V.—*Comparative optimum reaction and toleration limits of the clover bacteria, and Bacterium sojae and Bacterium glycineum.*

ORGANISMS, GROWTH, AND P_H CONCENTRATION ON SEVENTH DAY

Medium	Initial tests of media.			Clover, Virginia isolations.						Clover, Wisconsin isolations.				*Bact. sojae.*				*Bact. glycineum.*		Control.	
	Fuller's scale reading.	P_H reading.		Strain M.		Strain 3.		Strain 4.		No. 276.		No. 210.		Strain L.		Strain 8.		Strain No. 270		Fuller's scale reading.	P_H reading, colorimetric.
		Colorimetric.	Electrometric.	Growth.	P_H.	Growth.	P_H.	Growth.	P .	Growth.	P_H.	Growth.	P_H.	Growth.	P_H.	Growth.	P_H.	Growth.	P_H.		
BEEF (FRESH INFUSION) BOUILLON WITH 1 PER CENT DIFCO PEPTONE.	+30	5.5	5.5	+	6.6	+	6.8	+	6.6	+	6.8	+	5.9	−	.....	−	.....	−	.....	+35	5.5
	+27	6.0	5.9	++	7.0	++	7.2	++	6.8	++	7.1	+++	7.1	++	7.2	+++	7.2	+++	7.0	+30	6.0
	+22	6.4	6.3	++	7.1	++	7.4	++	7.2	++	7.4	+++	7.2	+++	7.1	+++	7.3	+++	7.3	+24	6.3
	+18	6.8	6.62	++	7.4	++	7.7	++	7.6	++	7.6	++	7.8	++	7.8	++	7.6	+++	7.6	+21	6.6
	+14	7.0	6.93	++	7.6	++	7.8	++	7.6	+	7.8	++	8.0	++	7.8	++	8.2	++	8.2	+16	7.0
	+10	7.2	7.26	++	7.6	++	8.0	+	7.6	+	8.0	+	8.0	++	8.2	++	8.4	++	8.2	+14	7.3
	+ 5	7.7	7.61	+	7.7	+	8.0	+	7.8	+	8.2	+	7.8	+	8.4	+	8.4	++	8.2	+7	7.7
	0	8.2	8.05	+	7.8	+	8.2	+	7.8	+	8.4	+	7.9	+	8.4	+	8.4	++	8.4	+4	7.8
	− 3	8.4	8.32	−	.....	±	.....	−	.....	+	8.4	+	8.2	+	8.4	+	8.4	++	8.4	+2	8.2
	− 5	8.8	8.63	−	.....	−	.....	−	.....	+	8.4	+	8.4	+	8.4	+	8.4	+	8.4	−1	8.4
	− 9	9.0	8.85	−	.....	−	.....	−	.....	+	8.6	±	8.4	+	8.6	+	8.4	+	8.4	−4	8.6
BEEF (EXTRACT) BOUILLON WITH 1 PER CENT DIFCO PEPTONE.	+28	3.4	3.43	−	.....	−	.....	−	.....	−	.....	−	.....	−	.....	−	.....	−	.....	+29	3.4
	+22	3.9	3.84	−	.....	−	.....	−	.....	−	.....	−	.....	−	.....	−	.....	−	.....	+24	3.9
	+17	4.4	4.35	−	.....	−	.....	−	.....	−	.....	−	.....	−	.....	−	.....	−	.....	+18	4.3
	+12	5.2	5.08	?	5.4	?	5.4	?	5.4	−	5.3	+	5.3	?	5.2	?	5.2	+	5.2	+13	5.2
	+ 8	6.7	6.7	++	7.6	++	7.8	++	7.6	+++	8.0	++	7.8	+++	8.0	++	8.0	+++	8.0	+7	6.7
	+ 3	7.8	7.87	++	7.8	++	8.4	++	8.2	+++	8.4	++	8.2	+++	8.4	++	8.4	+++	8.4	+4	7.7
	− 1	8.6	8.59	−	.....	−	.....	−	.....	++	8.8	+	8.4	++	8.8	++	8.8	++	8.8	−1	8.4
	− 6	9.5	9.09	−	.....	−	.....	−	.....	+	9.0	+	8.8	++	9.1	+	9.0	++	8.8	−4	9.0
	−10	10.0	9.39	−	.....	−	.....	−	.....	+	9.0	−	9.0	+	9.1	+	9.0	+	9.0	−7	9.4

− No growth. + Slight growth. ++ Moderate growth. +++ Abundant growth.
± A few tubes showed slight growth.
? A slight clouding in 3 days which disappeared before the seventh day.

In beef infusion media with a P_H value of 5.5 the soybean organisms produced no growth and the clover organisms did not grow until after three to five days and then only slightly. From the delayed and slight amount of growth, it is assumed that in beef infusion a P_H value of 5.5 represents about the limit of acid toleration.

Growth records made on third and fifth days after inoculation show that for all these organisms P_H values 6.4 to 6.7, in either beef infusion or beef extract media, were most favorable for production of early and continued growth.

TEMPERATURE RELATIONS

The organism grows well at a wide range of temperature. In one series of trials at Madison on this phase of the problem cultures on potato-agar slants and in bouillon were incubated at temperatures ranging from 3° to 39° C. at 3° intervals. In another, temperatures of 25°, 30°, 31°, 33°, 34°, 35°, 37°, and 38° C. were maintained. The parasite was able to make appreciable growth at 3° C. Its optimum in bouillon was 26°, whereas in agar 18° to 21° C. appeared to be most favorable. Slow but continued growth could be maintained at 34° C., but 35° inhibited development.

In determining the thermal death point in studies at Madison, two loopfuls of 48-hour-old broth culture were transferred to tubes of beef-extract-peptone broth. After 10-minute exposures in the usual manner the tubes were cooled and incubated. As a result of two series of trials, it was determined that the thermal death point lies between 48° and 49° C.

Experiments at Washington with the Virginia strains gave results entirely in agreement with these statements.

RESISTANCE TO DESICCATION

Vigorous broth cultures, 48 hours old, were diluted with water to twice the orginal volume, and drops of this suspension were allowed to dry on sterile cover glasses kept in sterile Petri dishes. After the drops had dried, tests were made by inserting the cover glasses into tubes of nutrient broth. No growth appeared after 30 minutes' desiccation, so that the organism is to be regarded as very susceptible to drying.

Tests in Washington with the Virginia strains gave the same results.

VITALITY ON CULTURE MEDIA

The clover organism has been found to retain a vitality on the usual culture media for an indefinite period, having been maintained in Wisconsin on potato agar without appreciable loss of vigor for about four years.

PATHOGENICITY

The type organism from red clover in Wisconsin has been found to hold its pathogenicity in agar culture for about one year but has tended gradually to lose it thereafter. The strains from the other localities have behaved similarly. The preliminary pathogenicity trials made at Madison, Wis., in 1916 included inoculations of red clover and soybeans, since it was then thought that the bacterial leafspot and soybean blight

might be indentical. Infections were readily secured upon red clover, but the inoculations of soybeans were unsuccessful. Other isolations including strain 1919–I, made in the summer of 1919, were successfully employed in infecting red clover in another series of experiments. Again, in 1920, a more extended series of tests of pathogenicity was instituted. These trials included the four clover species, *Trifolium pratense*, *T. medium*, *T. repens*, *T. hybridum*, and the white sweet clover, *Melilotus alba*. Four recently isolated strains of the organism, three from *Trifolium pratense* and one from *T. medium*, were used in these series. Water suspensions of 3- to 4-day-old potato-agar cultures were applied with an atomizer, after which the wetted leaflets were gently rubbed between the fingers. The inoculated plants were then covered from 48 to 72 hours with bell jars when the sky was clear, and were left uncovered if cloudy weather prevailed. Inoculated plants were sprayed once daily with sterile water to favor infection. Plants both out of doors and in the greenhouse were thus inoculated. After a period of incubation of 6 to 10 days, lesions appeared uniformly on all inoculated plants of *Trifolium pratense* and *T. medium*. Inoculated white clover, alsike clover, and white sweet clover, however, remained free from infection. The failures to secure infection of these species receive support from the field observations made in Wisconsin, since no cases of natural infection of these species or of alfalfa have been discovered, although all have been found growing closely intermingled with diseased red clover.

Several series of inoculation experiments have also been conducted at Raleigh, N. C., during the several seasons in which the investigations have been in progress. One of these, in the summer of 1922, is representative in all respects of the others and is, therefore, briefly described. Cultures from each of the clovers, red, white, and alsike, which had been grown for 48 hours on bacto-agar were used as an inoculum. The growth was washed off with sterile water and the bacterial suspension poured into Petri dishes. On the morning of July 22 inoculations were effected by immersing the leaves in these suspensions. The strain from any one of the host species was used to inoculate plants of that species and also the other two species. All inoculated plants were grown in the greenhouse and after inoculation were shaded lightly for 48 hours with newspapers. On July 29 small translucent areas, evident only on the lower leaf surface, were present in abundance on all inoculated plants. Three days later these lesions had developed into small, blackish brown spots characteristic of the disease in nature.

The same strains were also used in North Carolina to inoculate plants of soybean, but no evidence of infection appeared.

At Washington both greenhouse and out-of-door plants were used in the inoculation experiments, which extended through two years. Bacteria from 1- to 4-day-old-agar cultures were washed off in sterile water and this bacterial suspension was then sprayed on the plants with an atomizer. Usually the plants were covered with bell jars or placed in moist chambers for 24 to 48 hours after inoculation, then returned to normal conditions.

The first evidence of infection was noted in from 6 to 10 days as tiny, translucent areas which enlarged in a few days into the nearly black spots with translucent borders. Successful but slower infections resulted if the plants were not kept unusually moist for a period following inoculation. Check plants invariably remained free from infection.

Infected plants continued to develop new infections, but there seemed to be little or no spread of the infection to other plants not in direct contact with infected leaves.

All of the strains isolated at Washington produced infections not only on the original host species but also on other species, and in the numerous cross inoculations no difference was observed in the degree of pathogenicity on the various species. Strains from either red, white, alsike, or other species produced the characteristic lesions on any of the species. The following clovers were artificially infected: *Trifolium pratense* L., *T. pratense* var. *perenne* Host., *T. repens* L., *T. repens* var. *latum* McCarthy, *T. medium* L., *T. hybridum* L., *T. incarnatum* L., and *T. alexandrinum* L.

Lima bean (*Phaseolus lunatus*) and velvet bean (*Stizolobium deeringianum*) were also infected by the Virginia strains of the clover bacteria. On lima beans the infection was slight. The lesions were small, reddish, with a surrounding white zone and without any translucent tissue. On velvet bean the infection was moderate, lesions dark, almost black, circular to angular, and with translucent borders.

At Washington many attempts were made to infect soybeans (*Glycine hispida*). Three varieties—Ito San, Wilson Fine, and Black Eyebrow—were used in the tests. These were inoculated at various ages and with various strains of the clover bacterium, but no infections were ever secured. Similar plants used as controls were readily and typically infected with *Bact. sojae* and *Bact. glycineum*. The number and the thoroughness of these experiments on soybeans give evidence of definite specific differences between the clover and the soybean bacteria.

Two attempts to infect alfalfa gave negative results.

Further inoculation experiments will be necessary to determine how to interpret the differences in pathogenicity observed in the Mississippi Valley and the two seaboard stations. It may be that we are dealing with specialized races, the one restricted to the red clovers, the other having wider host range. It will be recalled that the Wisconsin strain 1920–III which was pathogenic to the red clovers in Wisconsin in the 1920 trial was found in 1921 in North Carolina to be culturally indistinguishable from the strains isolated in that State. It had, however, then lost its virulence even for red clover, indicating that pathogenicity is not a fixed and constant character with this species.

The results at all three stations are, however, in full accord so far as concerns the nonpathogenicity on soybeans. These failures to infect soybean with bacterial leafspot of clover furthermore substantiate the cultural studies in showing that the clover organism and the one from soybean are specifically distinct.

In the Washington laboratory comparative cultural tests, made with the Wisconsin and the Virginia clover strains, indicate that they are not identical in cultural characters. The tests were repeated several times and always gave the same results. These variations may be inherent or merely due to differing ages of culture. The chief cultural differences observed in parallel tests at Washington are summarized below.

VIRGINIA CLOVER BACTERIA.

1. Growth pure white and opaque on agar.
2. Growth extremely viscid on beef media.
3. No fluorescence in beef media.
4. Milk not coagulated. Remains opaque and cream color.
5. Strong reduction of litmus in milk.
6. Congo red in media containing dextrose is unchanged.
7. Potato agar, smooth growth.
8. Potato cylinders, slight growth.
9. In Uschinsky's solution, viscid pellicles and sediment and very slight fluorescence.
10. Casein not digested.
11. Approximate alkali limit, P_H 8.2 to 8.6.

WISCONSIN CLOVER BACTERIA.

1. Growth grayish white and not entirely opaque on agar.
2. Growth butyrous on beef media.
3. Fluorescent in beef media.
4. Milk coagulated. Becomes translucent and brownish.
5. Slight to no reduction of litmus in milk.
6. Congo red in media containing dextrose changed to dark purple-brown (acid reaction).
7. Potato agar, usually contoured growth.
8. Potato cylinders, abundant growth.
9. In Uschinsky's solution, pellicles and sediment not viscid, moderate flourescence.
10. Casein digested.
11. Approximate alkali limit, P_H 9.0 to 9.5.

TECHNICAL DESCRIPTION

Bacterium trifoliorum, n. sp.[10]

Cylindrical rods rounded at ends, solitary or in short chains; cells 1.2 to 3.0 μ by 0.4 to 1.0 μ, with an average length of 1.7 μ and a width of 0.7 μ; motile by means of one to four unipolar flagella; aerobic, Gram negative, no spores; not conspicuously capsulated.

Colonies on nutrient agar grayish white, glistening, margins entire, convex or slightly umbonate.

Gelatin not liquefied, nitrates not reduced, digests starch very feebly, acid formed from dextrose and saccharose, no gas produced in various carbohydrate media.

Group number 212.2322023, following the descriptive chart of 1917 of the Society of American Bacteriologists.

Type collected at Madison, Wisconsin, on *Trifolium pratense* L.

The type strain from Wisconsin has proved pathogenicity only on the red clovers (*Trifolium pratense* L. and *T. medium* L.); the strains from North Carolina and the vicinity of Washington, D. C., otherwise scarcely distinguishable, have been proved pathogenic also on *T. repens* L., *T. hybridum*, L., *T. incarnatum* L., *T. pannonicum* L., and *T. alexandrinum* L. Lesions occur on leaves, stems, petioles, stipules, and flowers.

Distribution apparently widespread in the northern Mississippi Valley on the red clovers *T. pratense* and *T. medium*, and the same organism, at least on the Atlantic seaboard, occurs on *T. repens*, *T. hybridum*, *T. incarnatum*, *T. pannonicum* and *T. alexandrinum*.

Specimens on all these hosts showing both natural and artificial infections have been deposited in the herbaria of the department of plant pathology, University of Wisconsin, and the Bureau of Plant Industry, United States Department of Agriculture.

RELATION OF PARASITE TO HOST TISSUE

Infection apparently occurs through the stomates. This has been evidenced by noting that lesions first appear on the lower leaf surface, the one occupied by the breathing pores. Then, too, young lesions have been fixed in alcohol, embedded, sectioned, and stained with methylene blue. The intercellular spaces of the mesophyll immediately subjacent to the stomates in such sections are seen to be densely packed with bacteria. In older lesions in which the host cells have collapsed, the parasite may invade the cell cavities.

[10] According to the classification of Migula and the recent report of the committee of the Society of American Bacteriologists the name of this clover organism would be *Pseudomonas trifoliorum*, n. sp.

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OVERWINTERING AND DISSEMINATION

No experimental evidence on the overwintering and dissemination of *Bacterium trifoliorum* is at hand. The field observations, however, during the several seasons in which the disease has been investigated show that it recurs annually in the same general areas in the clover fields and on the same small groups of plants in meadows and lawns. One old clover field at Madison was observed almost daily during April, May, and June, 1920. The disease appeared, with the advent of warm weather in April, upon the young leaves soon after they had unfolded. Here, no doubt, fallen diseased leaves harbored the parasite during winter and it spread from them to the new leaves. The disease was very much in evidence during May, and by blossoming time every leaf on a plant might be conspicuously spotted.

FIG. 3.—Diagram of two clover leaves in which insect injuries (*Phytonomus punctatus*) and the bacterial lesions (*Bacterium trifoliorum*) are associated. In the leaf at the left, the lesions were young and water-soaked and apparently had originated at the insect puncture. In the leaf at the right, the insects may, at least in some places, have eaten out the invaded tissue. In any case, the water-soaked margins indicated that the invasion was still progressing. (Drawing by Charles Drechsler.)

The fact that the disease under favorable conditions involves the plant so generally makes it highly probable that the floral parts might become infected. Lesions have not been observed, however, on the floral organs, but they are of common occurrence on the flower pedicels. Even though flowers are not involved, there would be ample opportunity for the seed to become contaminated either while yet in the field or during harvesting or threshing. The initial infections in newly planted fields could thus come from contaminated seed. That seed serves as the primary source of infection is indicated by the occurrence of diseased plants in newly planted pastures and lawns. Should this disease ever become seriously destructive, any precautionary measures looking toward its control or prevention, especially in new plantings, should stress the possible value of seed disinfection.

The most rapid spread of bacterial spot, as shown by field observations, occurs when there is an abundance of rain or dew. At these times conditions are most favorable for the spattering of the bacterial exudate to adjacent healthy leaves of plants, and opportunity is given for the bacteria to gain entrance to the leaves by means of the surface film of moisture.

Aside from rain and dew as agents of dissemination of bacterial leafspot, there is considerable observational evidence that certain leaf-eating insects, especially the larvae of the clover leaf weevil, *Phytonomus punctatus*, are responsible for its spread. Initial infections not uncommonly occur at the places where the leaves are injured by the feeding of these insects. In experimental feeding trials, clover leaf weevils avoided in every case eating other than the healthy tissues of abundantly spotted leaves offered them. The fact, however, that perforations made by weevils are the loci of infection indicates insect carriage. Many other plant pathogens whose normal mode of entering is through stomates are known to gain entrance also through wounds.

SUMMARY

(1) A hitherto undescribed bacterial leafspot disease has been observed on several species of clover, including *Trifolium pratense*, *T. medium*, *T. repens*, *T. repens* var. *latum*, *T. hybridum*, *T. incarnatum*, *T. alexandrinum*, and *T. pannonicum*. It is known to occur in Wisconsin, Iowa, Indiana, Virginia, Maryland, and North Carolina, and is probably widely prevalent.

(2) Leaves, stems, stipules, petioles, and flower pedicels are known to be involved, but lesions have not been observed on the floral organs.

(3) The spots may appear at any time throughout the growing season. The lesions on the leaves are at first minute, translucent dots which enlarge and become, at length, irregular, blackish-brown areas. These areas have a translucent border and the surrounding tissues are yellowish green. Mature leaves are perforated and frayed, due to the drying and falling of portions of the affected tissues.

(4) Under favorable moisture conditions, a milky white bacterial exudate is formed on the lower leaf surface. On drying, this becomes a delicate incrusting film.

(5) Bacterial leafspot is caused by an organism which is herein described as *Bacterium trifoliorum*, n. sp. It forms whitish colonies on nutrient agar, is flagellate, and forms acid from dextrose and saccharose. According to the Descriptive Chart, its group number is 212.2322023.

(6) With the type strain from Wisconsin, infection was secured only on the red clovers, but with the strains from North Carolina successful reciprocal inoculations have been made on the red, white, and alsike clovers. The Virginia strains also cross-infect successfully. The parasite is intercellular and apparently enters chiefly through the stomates.

(7) Field observations indicate that the dissemination of the disease is accomplished through the agency of rain or dew and of leaf-eating insects.

(8) It seems very probable that the organism is disseminated with the seed and that such contaminated seed in new plantings are in consequence the primary loci of infection.

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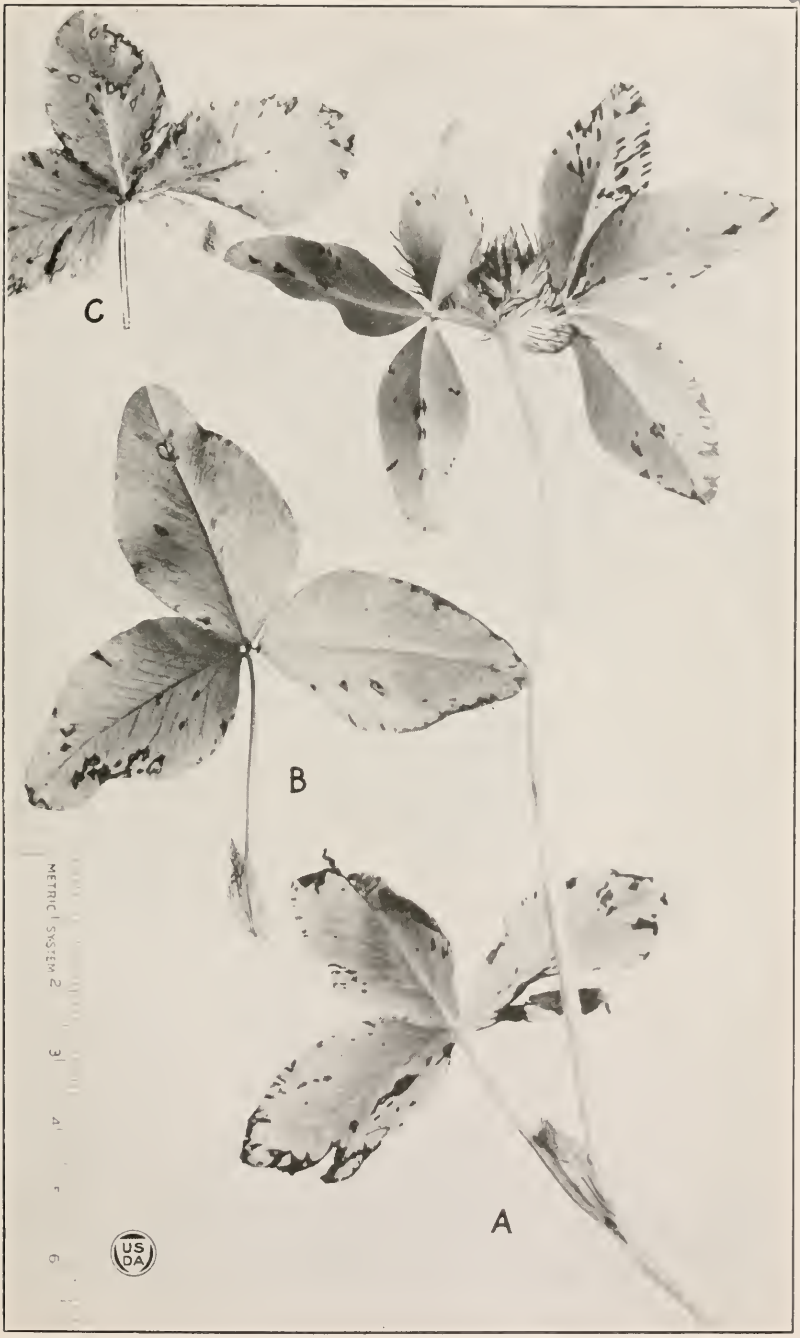
PLATE 1

Bacterial leafspot of red clover.

Blighted leaves of red clover (*Trifolium pratense*) from Madison, Wis., showing natural infection in various stages of development, collected by M. M. Williamson at Madison, Wis. Young lesions are characteristically distributed in the upper leaflets of branch A as well as in those of leaf B, while older lesions appear in the lower leaf of branch A and in leaf C. Note the uniform black color of the young lesions as contrasted with those in leaf C, in which the centers are dried out and lighter in color (cf. Pl. 2). The splitting of the dead areas following the drying out of marginal lesions, which causes a ragged appearance of the leaves, is well shown in the lower leaves of branch A and in leaf C.


C
B
A
METRIC SYSTEM

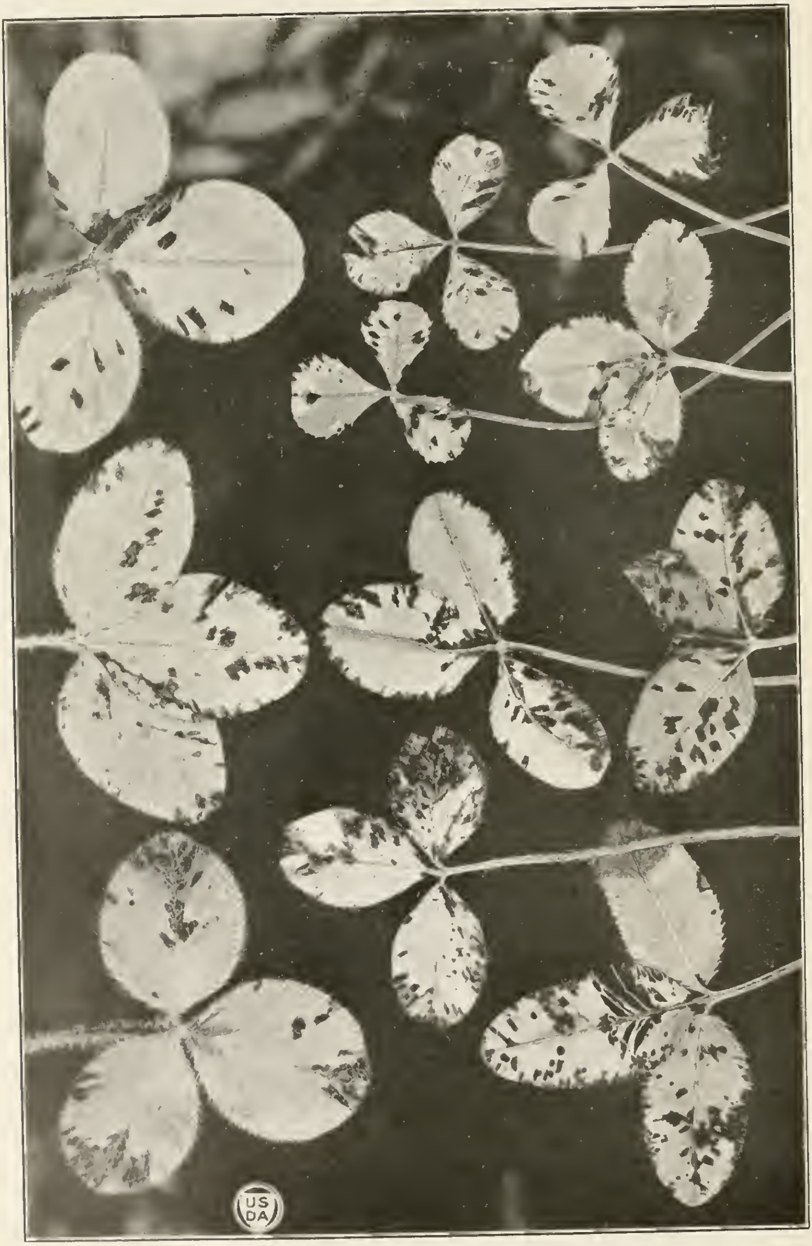


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PLATE 2

Bacterial leafspot on the three common clovers for comparison.

The disease is shown as it occurs on the three common clover hosts, natural infection, collected by F. A. Wolf at Raleigh, N. C. The three leaves in vertical alignment at the left are the red clover, *Trifolium prătense.* In the upper right-hand corner are grouped four leaves of the white clover, *Trifolium repens.* The remaining four, at the lower right hand, are the alsike clover, *Trifolium hybridum.* All natural infections.

PLATE 3

Bacterial leafspot of clovers.

A.—*Trifolium medium*, zigzag clover.

B.—*Trifolium repens*, variety *latum*, also known as Ladino clover. Photographed by transmitted light to show the translucent borders surrounding the lesions.

C and D.—Leaf and stipules of *Trifolium pannonicum*. The black streaks in the stipules are due to the bacterial infection.

Collected at Arlington, Va., by Lucia McCulloch. All natural infections. All natural-size photographs.

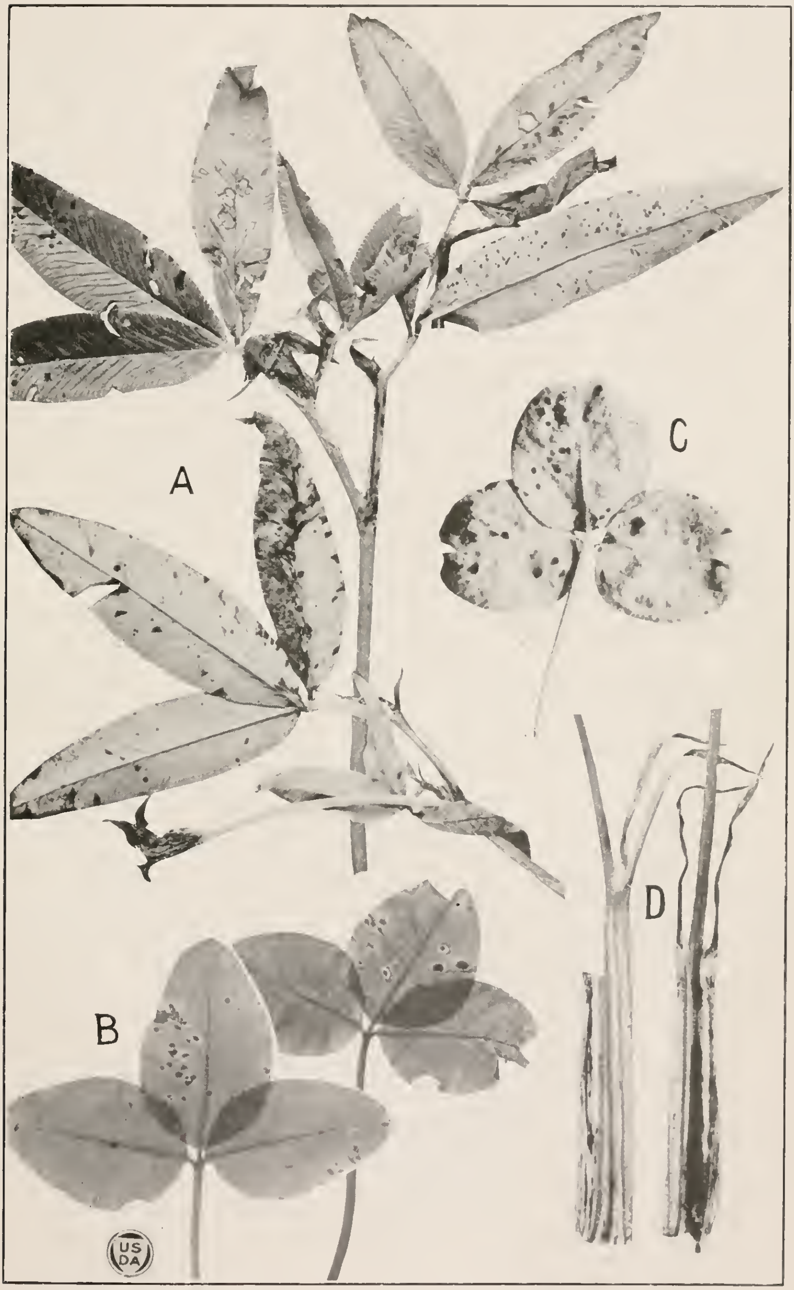
A
B
C
D

PLATE 5

Colonies of the clover organism.

Potato agar plate colonies of *Bacterium trifoliorum* incubated for 9 days at 26° C.

A.—Wisconsin isolation 1916–II, enlarged $\times 1\frac{1}{2}$, showing circular surface colonies and lens-shaped deep colonies. Note the rugose umbonate centers with radial corrugations extending outward toward the plain marginal zone. In very slow-growing colonies, these radial corrugations are commonly crossed by concentric folds. The plain zone at the margin is commonly raised, although less pronounced in some cases, as shown in B.

B.—Wisconsin isolation 1920–III enlarged $\times 1\frac{1}{2}$. The same colony characteristics appear in this strain as in 1916–II and other slower growing isolations. Indeed, the amount of growth may not be much greater, for the colonies seem more spreading and relatively thinner. Note, for example, the absence in colony B of the raised edge which is found in those of A. If this strain had not evidenced this spreading characteristic so constantly both in plates and tubes (Pl. 6) of the different agars used, it would seem to be an accident of moisture content. In their reactions in milk, sugar media, etc., the two strains, 1916–II (A) and 1920–III (B), seem identical; therefore these differences are attributed to variations in growth vigor and other minor or transient characters.

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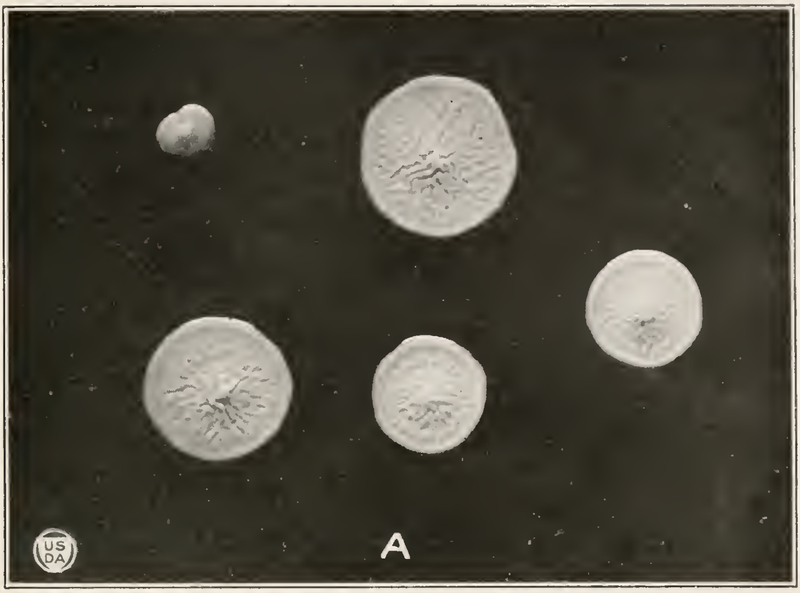

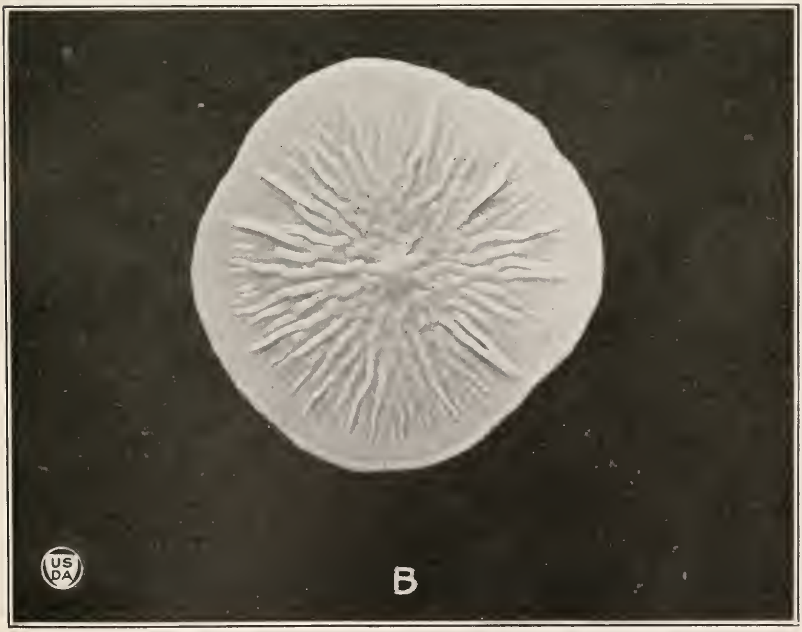


A
B

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PLATE 6

Agar slant cultures of the clover organism.

Potato agar (1.8 per cent agar) streaks of *Bacterium trifoliorum* incubated for four days at 26° C.

A.—Wisconsin isolation 1920–III illustrates well the characteristics of this more spreading strain. As in the plate colonies (see B, Pl. 5) the radial folds are not so closely packed, the edges of the colony less raised than in isolation 1916–II shown in B.

B.—Wisconsin isolation 1916–II. (Compare also with colonies in Pl. 5, A.)

A NEW TYPE OF ORANGE-RUST ON BLACKBERRY[1]

By B. O. Dodge

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The writer in discussing the distribution of the orange-rusts of Rubus[2] referred to certain blackberries obtained at Forestville, Md., in 1922. Some of the plants were infected with the typical short-cycled form, one plant with the long-cycled Gymnoconia, and two plants with a form whose spores were reddish orange and corresponded in other particulars to aecidiospores of the long-cycled rust. When spores from these two particular plants were sowed on agar, they germinated so quickly and with such long germ tubes that the rust was at first marked as long cycled. Examination of the plates a day or two later, however, disclosed large numbers of long promycelia bearing sporidia. On repeating the germination test, the same results were obtained. Any long germ tubes that persisted were ignored, or assumed to be abnormal. The presence of hundreds of promycelia could not be overlooked. The appearance of the rust being so like that of the ordinary Gymnoconia and the manner of spore germination so puzzling, a more critical study of this strain of the rust was made in 1923, when the plants were in far better condition for spore production than they had been just after being transplanted the previous year. The results of the later investigation show that three plants are infected with what might be called an intermediate form, because some aecidia are short cycled and others long cycled.[3] It is interesting to find here an example which can be best described as a short-cycled rust being derived from a long cycled form. On April 11, 1922, 12 infected wild blackberries, No. 288–299, were dug up in a pasture at Forestville, Md.; two plants, No. 290 and 294, died soon afterwards. A brief account of this collection of rusted plants may serve to bring out more clearly the true nature of our Rubus orange-rusts.

If one plant is infected with the long-cycled rust, and another with the short-cycled, aecidia will usually, other conditions being equal, mature about a week or 10 days earlier on the latter plant. After the 10 surviving plants in this collection were brought to the greenhouse from the cold frames, March 10, 1923, the date of the maturing of the first aecidia on each was noted. Within three weeks aecidia had matured on six plants. The aecidia on five plants were yellowish orange in color, and their spores produced regular promycelia. This is certainly our common short-cycled blackberry rust. The rust on No. 297 needs further study. About 10 days later aecidia matured on the other four plants. Germination tests were made of spores from several leaves from No. 291 and no promycelium was found. The rust on this plant is typically long-cycled, and is of no particular interest in itself.

It was not until we had improved our methods of testing spore germination that we discovered that the rust on the other three plants was

[1] Accepted for publication June 25, 1923.

[2] Dodge B. O. THE DISTRIBUTION OF THE ORANGE-RUSTS OF RUBUS. *In* Phytopathology, v. 13, p. 61–74. 1923. Literature cited, p. 74.

[3] For convenience may we not refer to an aecidium as "short cycled" if its spores produce promycelia, and as "long cycled" if they produce germ tubes on germination?

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producing two sorts of aecidia. Spores will germinate normally if floated on water or placed in hanging drops, but agar plates are far more convenient and satisfactory for this work. The writer's method now consists in placing a leaf bearing young aecidia on agar in Petri dishes so that spores from individual sori fall together on the surface of the agar as they are naturally shed. A spore print is obtained in a few hours, showing the location of the ruptured sori on the leaf. The same leaf can be used several times to obtain additional spore prints for study. The strongly negatively heliotropic reaction which sends the germ tubes down into the agar will be avoided if the plates are more equally illuminated from below and above. The temperature factor need not be considered in this connection so long as the spores germinate.

A SHORT-CYCLED RUST IN THE MAKING?

As soon as spore prints of individual sori on leaves from plants No. 292, 293, and 296 had been obtained, it was clear that the spores from all sori had not been discharged in the same way. Certain aecidia had shed spores that were very dry or powdery, so that they had been evenly scattered like dust on the agar. The spores from other aecidia seemed to be more waxy and tended to cling together, falling in little clumps, like waxy pollen. The dusty spores were shed from the sorus as soon as mature; the waxy spores tended to stay in the sorus and pile up in irregular masses. It was found that the spores in the waxy sori produced promycelia, while the more dusty spores produced only long germ tubes. Contrary to what one would expect, the waxy sori (short cycled) were of a more reddish orange, while the others (long cycled) were more of a golden orange. The color contrasts showed very distinctly in the spore prints. Considering, then, the waxy nature and the color of the spores, it was not at all difficult to tell by examining a sorus with a hand lens how its spores would germinate.

DISTRIBUTION OF AECIDIA ON THE LEAVES

The leaves on these blackberries have three leaflets. Sometimes all aecidia on the three leaflets will be long cycled, while those on another leaf will be short cycled. Again, all sori on the terminal leaflet will be short cycled, while those on the other two are long cycled. It was frequently noted that aecidia at the base of a leaf or leaflet were long cycled and those toward the tips short cycled, but no general rule seems to be followed in this distribution. Occasionally, a long aecidium was found in which the spores at one end were long cycled and those at the other were short cycled. This might have been caused by the running together of separate sori. The writer has had in mind the task of infecting the same blackberry systemically with both the long-cycled and the short-cycled forms of orange rust. Such an experiment would probably necessitate the development of teleutospores of the Gymnoconia in the greenhouse so that they could be sowed on new shoots of the blackberry at the same time that the aecidiospores of the short-cycled rust were being matured in nature. This would mean that the greenhouse work should begin about six weeks before the orange rust appears in the field. Local gametophytic infections could be affected so that the mycelium of each type would remain isolated in different nodes, to run together at some common node. Should the two mycelia come together in a leaf,

conditions would be right for hybridization. It was at first thought that such a type of infection had actually occurred in nature, thus accounting for the two kinds of sori in the three plants mentioned. The evidence, however, is against such a supposition. The writer has recently obtained a number of blackberries not only from Forestville, but from other localities, in which the rust was maturing two kinds of aecidia. The typical short-cycled rust has waxy, yellowish orange spores, but the short-cycled sori in plant No. 292, for example, are of an even darker and more reddish orange than are the long-cycled sori from the same leaves, and the spores also correspond in shape and size to spores of the typical long-cycled form. If we had two mycelia in the leaf the short-cycled *Caeoma nitens* type should be yellowish orange. The chances that teleutospores of the Gymnoconia and aecidiospores of the short-cycled form would mature at the same time in a given locality are rather remote. The writer has, however, collected good orange-rust aecidia in September. The seasonal conditions which would ordinarily bring out the orange-rust stage of one form would be just as favorable for the development of that stage of the other form. The time intervening between the sowing of the aecidiospores of the Gymnoconia and the development of its teleutospores is at least one month, and is usually much longer. Aside from the old doctrine of immutability of species, the best evidence that two mycelia are actually present in these leaves is the fact that the two types of aecidiospores are generally borne in separate sori.

Several blackberries have been inoculated with aecidiospores from No. 292, 293 and 296. Teleutospores have already matured. We shall wait with interest until next spring to learn, in case there is systemic infection, whether or not the new rust will develop two sorts of aecidia. If it does it will have been proved that this phenomenal development of two kinds of aecidia is not due to the presence of two different orange-rust mycelia in the same leaf, but is due rather to the unstable nature of this particular form.

The writer stated in a recent paper [4] that if one should visit Schweinitz's old collecting ground at Salem, N. C., about May 15, he would probably find the long-cycled Gymnoconia on blackberry. The opportunity was afforded the writer himself to make this trip May 19, 1923, and he had no difficulty in picking up any number of specimens of rust on blackberry which by color and test by spore germination were proved to be long cycled. Just which rust Schweinitz [5] had, however, when he described *Caeoma nitens* is far from a certainty. The Gymnoconia was also found to be very common at Cornelia, Ga., May 17. Teleutospores will be developed on blackberry here about August.

The short-cycled rust on dewberry in more southern areas is a well-fixed form, morphologically distinct from the Gymnoconia on black raspberries and on *Rubus saxatilis*, of Europe and Asia. The fact that one form is maturing two kinds of aecidia, as described above, may indicate that a third type is arising. The aecidiospores are far more like those of the long-cycled Gymnoconia. The evidence based on distribution also favors the belief that the long-cycled orange-rust is the more primitive. Only this type occurs in Europe and Asia, and it is now known over most of North America, probably extending as far south and west as the short-cycled form. The westerly winds might well have

[4] Dodge, B. O. op. cit.
[5] Schweinitz, Lewis David von. synopsis fungorum carolinae superioris . . . ed. a D. F. Schwaegrichen. 105 p., 2 col. pl. 1822. E. Commentariis. Societates naturae curiosorium lipsiensis excerpta.

spread the Gymnoconia, with its somewhat dry and dusty spores, from western Europe through Russia into Siberia and across the straits to Alaska. From there it probably followed the coast and crossed the continent from west to east and from north to south. Many additional susceptible species of host were met in America, at the same time that widely different climatic conditions were encountered, all of which favored a change of life habits by the rust, which culminated in the elimination of the teleutosporic stage entirely. Until some one has secured systemic infection of blackberry directly by sowing aecidiospores from the raspberry, which Kunkel says sometimes produce promycelia, and has obtained a systemic rust at once comparable to our common short-cycled rust, the writer sees no valid reason why separate specific or even generic names might not be applied to two forms which are well fixed. The third or intermediate type which is maturing aecidia of two kinds may represent a strain of Gymnoconia which is particularly unstable and from which a short-cycled rust is now arising and which will have distinct morphological characters of its own.

As the writer understands Kunkel's [6] conception of the two life cycles involved in the Gymnoconia, the aecidiospores are all alike in that, especially if germination can be delayed by lowering the temperature, a few normal as well as abnormal promycelia are apt to be found in almost any germination test. This may very well be the true nature of the common Gymnoconia, but the form of the rust which produces two kinds of aecidia is in an entirely different category. In this form the nature of the aecidiospore, whether it is to produce a germ tube or a promycelium, is predetermined as it is being matured. The fusion in the spore of its two nuclei might very well be accompanied by changes in the coloration of the spore contents and in the nature of the spore wall, but by no kind of treatment during germination could a normal aecidiospore germ tube be obtained. On the other hand, precocious nuclear fusion, either in the spore or in its tube, might be induced by some external treatment so that a promycelium with sporidia would follow. In case such sporidia were capable of infecting the host, we should expect the rust to go back to its former habits in the new generation resulting from this infection. The nature of the germ plasm is believed not to be easily and permanently altered by environmental changes.

The writer has given reasons for believing that the production here of two kinds of aecidia on the same leaf is due to the variable nature of a strain of the Gymnoconia which normally includes the old *Puccinia peckiana* in its life cycle.

Whatever may be one's view regarding the manner of the origin of a species, and whether in this case we say a species is arising as the result of hybridization or by mutation, it is clear that the practice of applying the terms telium and teliospore to the aecidium and aecidiospore of a short-cycled rust just because the aecidiospore happens to produce a promycelium instead of a germ tube can no longer be defended. Such terms should represent morphological units, and not behavior. No doubt a study of the individual aecidia of a number of other rusts which were shown by Jackson at the Boston meeting of the Botanical Society of America to have correlated short and long cycled forms will bring forward additional evidence to prove that what we are pleased to call species are arising to-day as they have arisen for ages past.

[6] KUNKEL, L. O. FURTHER DATA ON THE ORANGE-RUSTS OF RUBUS. *In* Jour. Agr. Research, v.19, p. 501-512, pl. D, 92-94. 1920. Literature cited, p. 512.

EFFECT OF THE ORANGE-RUSTS OF RUBUS ON THE DEVELOPMENT AND DISTRIBUTION OF STOMATA[1]

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In order to obtain a supply of teleutospores with which to carry on some experiments on infecting the black raspberry (*Rubus occidentalis*) with the gametophytic stage of Gymnoconia, the writer has frequently sowed aecidiospores when the topmost leaves of new canes were still folded, while the lower leaves and all those on old canes were fully expanded. Although many trials, both in the field and in the greenhouse, have resulted in failure to obtain infection, the most abundant production of teleutospores followed inoculation of leaves of the latter type. It has been shown repeatedly that aecidiospore germ tubes gain entrance into a leaf through the stomatal openings. Clinton[2] reports that the stomata of leaves of blackberries and raspberries are confined to the lower surface except for a few which occur along the margin on the upper side. He believes, therefore, that infection by aecidiospores of the orange-rust must occur at a time when the side bearing the stomata is exposed to receive the spores falling from above, which would be when the leaves are beginning to unfold. The ventral surface is then turned outward and somewhat upward, and the halves are so folded together as to expose the margins perfectly to catch the spores. As the results of our experiments did not strongly support the theory that leaves are the most readily infected before they unfold completely, a study was made of the development and distribution of stomata on various types of leaves from blackberries and raspberries. The fact that agitation by the wind and the work of insects might well carry spores to the underside of fully expanded leaves would not minimize the importance of the distribution of stomata in facilitating infection. In the course of this work it was found that the invasion of leaves by the orange-rust mycelium has a very unusual effect on the production of stomata.

If account is taken of the conditions of the leaves of our blackberry or raspberry in nature at the time aecidiospores are being shed, it will be seen that several types are exposed to infection. The first leaves to unfold are the ones on the normal old canes; the infected old canes are also developing their leaves at this time. Practically all leaves on the old canes, normal and infected, are of about the same age. New shoots from the base of infected and of uninfected plants grow up a little later. During the growth period of the new canes there will always be leaves just unfolding at the top, and others lower down fully expanded.

In general, most of the stomata on normal leaves of Rubus are on the lower side, as stated by Clinton. A number of species of this genus have a few on the upper side at the tips of the serrations. Some blackberry leaves examined have a small number irregularly scattered, singly or in groups of two or three, on the upper side, especially along the larger

[1] Accepted for publication June 25, 1923.

[2] Clinton, G. P. orange rust of raspberry and blackberry. Ill. Agr. Exp. Sta. Bul. 29, p. 273–300, 4 pl. 1893. Literature, p. 292–295.

veins; in every such case the number is so small as to be of little consequence in the economy of the normal plant. It is quite otherwise in case of plants systemically infected with either of the orange-rusts.

Sections of leaves collected at random for another purpose from a systemically infected blackberry in New Hampshire show fully twice as many stomata on the upper side as they do on the lower side. An examination of sections of black raspberry leaves which had been prepared for a study of young aecidia revealed an abundance of stomata on the upper side. Further investigations disclosed the fact that the systemic stage of these orange rusts regularly so affects the host as to lead to the development of a large number of stomata on the upper side of the leaf where normally there would have been only a very few or none at all. One frequently sees that in a blackberry which has harbored the rust for two years or more most of the leaves on the old canes infected are evenly covered with spermogonia from the time they begin to unfold. From one-fourth to one-half of the total number of stomata on such leaves will be found on the upper side. Certain specimens of infected mountain blackberry did not show such a large percentage of stomata on the dorsal side; the increase in the number due to the stimulus of the rust was very marked. The development of spermogonia on leaves of primarily infected blackberries, to be noted later, is frequently inhibited, aecidia alone being formed. Neverthless, by noting the areas where stomata occur in large numbers on the upper side one can tell several days in advance just where aecidia are to be developed. Whenever the rust at maturity covers only a part of the leaf it will be found that only that part is provided with additional stomata on the upper side. As long as the epidermis is in a plastic condition the advance of the mycelium into new areas is accompanied by the development of additional stomata.

The effect of the parasite on the production of stomata varies with the course of development of the rust. Under certain conditions, spermogonia may be distributed uniformly over the leaf from the first, but the formation of aecidia is long delayed or is entirely omitted. There will be about the same number of stomata on both sides of the leaf, the normal number occurring on the under side. It has also been found that when certain blackberries show the rust for the first time after primary infection by sowing sporidia of the short-cycled rust, the spermogonial stage is entirely lacking. The infected leaves show by their yellowish-green margins the extent to which the rust hyphae have penetrated. Aecidia will later develop along the discolored margins, then gradually spread toward the midrib, preceded by the fading out of the natural green and the production of stomata on the upper side. Very few spermogonia were found on some 50 plants of the varieties Kittatinny and Iceberg the first two years following infection; aecidia developed normally. Other varieties, Ancient Briton, Blowers, etc., developed spermogonia under similar conditions. The complete suppression of this stage in the life cycle of a rust is said to be of rare occurrence. A further study of the rust on different varieties of blackberry may serve to suggest a reason for the incomplete development or nonproduction of spermogonia by other rusts such as the one on the mallows.

In rare cases aecidia also fail to appear, most of the leaf merely becoming yellowish. Such leaves, though never bearing any spore forms, have been invaded by mycelium, as shown by the large number of stomata on the discolored areas of the upper side, the usual number appearing below.

The gametophytic mycelium of Gymnoconia does not always penetrate into every part of the leaf of, for example, the black raspberry. One-half only may be infected; variegations occur or angular patterns are laid out, the space between certain large veins escaping attack. Photographic prints can be made directly from these leaves, either before or after the chlorophyll has been extracted (Pl. 1). It will be found that production of stomata on the upper side of the leaf is coincident with the invasion of an area by the orange-rust hyphae. Plate 1, C, shows that the infected area of the raspberry leaf is sharply limited on the left side by the vein, and toward the tip on the right side the mycelium has spread out part way between the second and third veins. There were no stomata on the dorsal side of this leaf, except over the infected areas. A somewhat different pattern was worked out in the leaf shown as Plate 1, A. Dorsal stomata occurred on the left side except at the base, into which

FIG. 1.—Relative numbers of stomata on corresponding areas from the ventral and dorsal sides of a leaf from an old cane of *Rubus occidentalis* infected with the long-cycled orange-rust. Position of stomata and spermogonia determined with the aid of a camera lucida: size and shape of stomata purely diagrammatic. In fact, the stomata on the dorsal side are on the average somewhat larger. Circles represent positions of spermogonia. The fields diagramed are such as were covered by a 16 mm. lens and Leitz Periplane 10x ocular. A, Area from ventral surface; B, Similar area from the dorsal side nearly opposite the area shown in A. There were 150 stomata in the area from the ventral side, and 136 in the dorsal area opposite. It would be necessary to examine several such fields before a single stoma would be found on the dorsal side of an uninfected leaf, or of an uninfected part of this same leaf.

hyphae had not penetrated. The lighter areas on the terminal and left lateral leaflets (Pl. 1, B), represent portions invaded and where dorsal stomata were numerous. The leaflets which escaped infection had no stomata on the upper side. Plate 1, F and G, shows prints of leaflets of Kittatinny blackberry on parts of which aecidia were just maturing. Practically the same number of stomata was found on the upper and lower sides on areas infected.

The time intervening between the opening of a particular infected leaf on new canes and the maturing of aecidia on that leaf becomes progressively shorter as the season advances, until a time is reached when fully formed aecidia are exposed as the leaf opens. What should be the effect of such an early appearance of aecidia on the underside of the leaf? The development of stomata on the underside is, just as we might expect, greatly interfered with, there being only a few normal stomata on that side, and these are at the tips of the serrations upon which no aecidia appear, just the reverse of the distribution of stomata in the normal leaf. An accurate count of the stomata has not been made on any considerable number of areas of normal and of infected leaves, but it is certainly inter-

esting to note that soon after the period is reached in a vigorous blackberry shoot when mature aecidia appear with the unfolding of the leaf, the tip of the new cane begins to outstrip the parasite in the upward growth, and the production of aecidia ceases; the grower says, "the plant is recovering."

That it is the gametophytic mycelium and not the sporophytic that has the power of inducing this morphological change in the host leaf is clear from a comparison of two sets of leaves, each bearing teleutosori. One collection was made July 10 at Lakewood, N. H. The leaves were thickly covered with spermogonia accompanied by an abundance of teleutosori, the aecidial stage having been suppressed. There were about the same number of stomata on both sides of the leaf. The other collection was made September 6 in Maine; these leaves, of course, bore only teleutospores. There were no stomata on the upper side, while the lower side was provided with what seemed to be the normal number. The occurrence of stomata on the upper side of leaves bearing spermogonia or aecidia could no doubt account for the infection of these leaves by aecidiospores. The two sorts of mycelium, systemic gametophytic and local sporophytic, not being antagonistic, develop their own reproductive bodies, spermogonia and teleutospores, side by side.

It has frequently come to the writer's attention that some of the earliest and most abundant development of teleutospores has occurred on leaves of old canes. These leaves must have been fully formed by the time aecidiospores were shed; this is especially true of systemically infected plants. Mountain blackberries harboring the orange-rust were transplanted from Maine to Maryland. In August, 1921, the leaves on the "new" canes bore an abundance of teleutosori, the "old" canes were defoliated and dying at this time. Early in June of the following year practically every leaf on the old canes bore teleutospores. These plants were watched again in 1923. Teleutospores first appeared on leaves of old canes which had previously borne spermogonia and frequently also aecidia. The position of the leaf on the cane, whether at the tip or at the base, was clearly of no great importance. The leaves on the new canes remained free from the telial stage during the summer of 1922. All leaves on the old canes had unfolded at about the same time and had borne aecidia, and would have been equally susceptible so far as maturity is concerned. On the other hand, leaves develop on the new canes one by one, as previously noted, so that as aecidiospores are shed leaves of different ages would be exposed. In this particular case in 1922 either the leaves on the new canes had not opened or they were not sufficiently mature when the spores were shed.

After seeing that leaves harboring the gametophytic mycelium were provided with additional stomata on the upper side, it occurred to the writer that this might account for the production of telia on so many leaves of the old infected canes. Aecidiospores were sowed on the upper side of leaves bearing spermogonia, the leaves then being placed in damp chambers. After two days the leaves were dropped in Flemming's fixture. The germ tube grows along the surface until it comes into the immediate vicinity of a stoma, then, if necessary, the end turns sharply, broadens out and sends the infection tube through the opening. The method of penetration was observed without sectioning by removing the chlorophyll from leaves killed in the Flemming's fluid. There were in the greenhouse on April 18 a number of potted plants of *Rubus occidentalis* systemically infected with the Gymnoconia, and now showing spermogonia. On many leaflets, especially on old canes, spermogonia

occurred only on a part of the leaf (Pl. 1, A). Aecidiospores were sowed over several plants in such a way that most of the spores fell on the dorsal side of leaves which had fully expanded. Many spores must have come in contact with the underside. The leaves on the tips of young basal shoots which had not unfolded were tagged. Particularly large numbers of spores were sowed on these folded leaves. The experiment was repeated April 25 with other plants. On May 25 teleutosori in abundance were found on plants from both sowings, showing that it takes at least four or five weeks in the greenhouse for the teleutosporic stage to reach maturity. Some of the leaves now bearing teleutosori also bore aecidia and spermogonia, while others bore only spermogonia; teleutospores were more abundant on such leaves.

In case aecidia or spermogonia occurred on only a part of a leaf, it was found that teleutosori were present only on that part, often on the upper as well as on the lower side. The part of the leaf not invaded by the gametophytic mycelium and free from the orange-rust stage had escaped infection when aecidiospores had been sowed on its dorsal surface. Leaves bearing teleutosori were decolorized and examined for stomata. The normal number appeared to be present on the ventral side of those leaves bearing only spermogonia and teleutosori. It was, of course, impossible to determine what had been the distribution of stomata on those areas on the ventral side of leaves where aecidia had destroyed the epidermis. Stomata were always found in abundance wherever teleutospores were present. Where aecidiospores of the Gymnoconia had been sprayed on both sides of leaves of old canes systemically infected, teleutosori first appeared on parts of leaflets having spermogonia. About 10 days later they began to appear among the hairs on the under side of neighboring leaves that had escaped invasion by the orange-rust hyphae. The results point clearly to the value of stomata on the upper side of leaves in facilitating the attack by the sporophytic germ tube. The gametophytic mycelium of the rust stimulates the host to provide ready means of access by the sporophytic stage which is to follow later.

As previously noted, the leaves of the black raspberry which are exposed to infection as the aecidiospores are being shed, may be grouped as follows:

1. Fully expanded but somewhat dwarfed leaves on old canes already infected with the systemic or orange-rust stage. The leaves or parts of leaves into which the gametophytic hyphae have penetrated will be rather devoid of hairs on the lower surface and have an abundance of stomata on the dorsal side, two factors favoring infection by the germ tubes.
2. Large, fully unfolded leaves on infected basal shoots from systemically infected plants such as produce the old canes in No. 1. Gametophytic hyphae evenly distributed throughout most of the leaflets, which will later usually be covered with aecidia. Very few hairs on the lower side, many stomata on the dorsal side.
3. Fully expanded leaves on normal old canes. Leaves somewhat tomentose on the underside. No stomata on the dorsal side (a very few at the tips of the serrations).
4. Very young leaves at the tips of systemically infected basal shoots. Some of these leaves will be tightly folded, others just expanding.
5. Fully expanded and growing leaves, tomentose beneath, on normal basal shoots.
6. Very young leaves, some still folded and very tomentose, others expanding, on basal shoots of normal canes.

The infection experiments described above show that some of the factors which determine the readiness with which a leaf of the raspberry can be infected by sowing aecidiospores resulting in the development of the teleutosori (*Puccinia peckiana*) are: Presence or absence of stomata

on the dorsal side, the maturity of the leaf, and the amount of tomentum covering the stomata. Leaves such as would come under groups 1 to 3 above are certainly most easily infected, and in the order given. Owing to the fact that it takes teleutosori a much longer time to mature on leaves of normal new canes, one is apt to be misled as to the relative susceptibility of the very youngest leaves. Very few teleutospores were ever developed on leaves which were still folded when the sowings of aecidiospores were made, even though these leaves were systemically infected, and therefore devoid of tomentum. The same line of infection experiments has also been carried on with blackberries and dewberries, with the same results. Teleutospores appear first and most abundantly if aecidiospores are sowed on leaves of the old canes that are systemically infected. The comparatively short time (four or five weeks) required for the development of teleutosori on leaves already harboring the orange-rust stage is accounted for by the rapidity with which such leaves reach maturity and fall off. The ease with which they may be infected is at least in part due to accessory dorsal stomata and freedom from tomentum.

Blodgett [3] reported that he had observed that blackberries bearing orange rust wilted sooner than uninfected plants, and that this was due to excessive transpiration.

Reed and Crabill [4] think that the greater loss of water by the rusted plant reported by Blodgett was due to the rupture of extensive areas of the ventral epidermis, which would facilitate evaporation. "Possibly other factors connected with the diseased condition may also operate to cause increased transpiration." These authors found that leaves of apples affected with Gymnosporangium transpire about the same whether they are in daylight or darkness. Healthy leaves transpire much more rapidly in daylight. The average for daylight and darkness, however, is practically the same for diseased and for healthy leaves. They think that the parasite must in some way affect the operation of the stomata. The substomatal cavities of rusted apple leaves are obliterated. Their figure 13 shows stomata only in the ventral epidermis of an area bearing spermogonia and aecidia.

In an isolated plant infected with Gymnoconia the chances for the production of telia are certainly vastly increased should the leaves which will some day have their ventral surface covered with blister-like aecidia be provided with stomata on the other side. Just this occurs as, we might say, a matter of safety, if not always of necessity, when leaves are systemically infected. Aecidia frequently fail to develop in leaves covered with spermogonia. In such a case there is no apparent reason why stomata on the ventral surface should not function properly. The accessory dorsal stomata are formed as the result of the stimulus of the gametophytic mycelium present just beneath the epidermis. The failure to produce aecidia following spermogonia is undoubtedly due to the condition of the host leaf, but it is not a condition under the control of the host. A great many investigations of the development and distribution of stomata have been made in the past, but the writer has been unable to find in the literature an account of another such curious interaction between host and parasite. There are several systemic rusts known which attack herbaceous plants. It would be interesting to know whether additional dorsal stomata are also developed by these hosts.

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[3] Blodgett, Frederick H. transpiration of rust-infested rubus. *In* Torreya, v. 1, p. 34–35. 1901.

[4] Reed, Howard S., and Crabill, C. H. the cedar rust of apples caused by gymnosporangium juniperi-virginianae schw. Va. Agr. Exp. Sta. Tech. Bul. 9, 106 p., 23 fig. 1915. Bibliography, p. 104–106.

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PLATE 1

The prints were made by bringing the leaves, from which the chlorophyll had been removed, in direct contact with Velox paper.

A.—Black raspberry leaflet, dorsal side. One-half of the leaflet, except a small portion of the lower left, infected and bearing spermogonia. Stomata on the upper side only where hyphae are present.

B.—Leaf of black raspberry, ventral surface. Left half of terminal leaflet infected; hyphae are beginning to invade the right half. Most of the left basal leaflet is infected; dark area along the midrib devoid of hyphae. No stomata were found on the dorsal side of the right basal leaflet, which was not infected, and none on the upper side of the others except where infected.

C.—Black raspberry, ventral surface. At the left the boundary line between infected and uninfected areas is very clear cut, being limited by a large lateral vein. On the right side the mycelium is advancing from the margin into the regions between the second and third veins; stomata present on the dorsal side only where leaf is infected.

D and E.—Leaflets from the same plant one month later; that is, one month after sowing aecidiospores of the Gymnoconia on the upper side of these leaves. Dorsal stomata and teleutosori only on areas now bearing aecidia. Teleutosori also on ventral side among aecidia.

F.—Leaf of Kittatinny blackberry aecidia maturing. About the same number of stomata were found on both sides of the portions of the leaflets bearing aecidia. No stomata on dorsal side of basal leaflet at the right.

G.—Larger leaflet of Kittatinny blackberry, showing that the fungus advances from the margin toward the midrib. Dorsal stomata only on the area where aecidia are present on the ventral side. No spermogonia were formed.

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